Effect of Far-red Light and Its Interaction with Red Light in the Photoperiodic Response of Pharbitis nil[1, 2]

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Red light given in the inductive dark period inhibits flowering in short day plants, and this effect may be reversed by far-red light given shortly after the red irradiation. The pigment system involved in these reactions is called phytochrome. In *Pharbitis,* however, the flower-inhibitory effect of red light is not reversed by succeeding irradiation with far-red (6, 7, 8, 13). The flowering response of *Pharbitis* is inhibited by far-red light given at the beginning of the dark period, and this inhibitory effect is completely reversed by red light applied shortly after the far-red irradiation (6, 7). On the other hand, in *Xanthium* far-red light given at the beginning of the dark period promotes flowering and shortens the critical dark period by some 2 hours (1). Takimoto and Ikeda (14), working with *Pharbitis,* found that far-red light given at the beginning of the dark period slightly promoted flowering when the dark period was shorter than 13 hours but inhibited flowering when the dark period was longer than 13 hours. It was also reported that far-red interruptions applied in a long dark period inhibited flowering when given in the first 16 hours of the dark period (13). Maximum inhibition was obtained when the far-red light was given 8 hours after the beginning of the dark period. Nakayama et al. (8) suggested that the inhibitory effect of far-red light given at the 8-hour point is a result of absorption by Pr (red absorbing form of phytochrome) rather than Pfr (far-red absorbing form of phytochrome). On the other hand, from the fact that both red and far-red light inhibit flowering at the 8-hour point, Salisbury (9) pointed out the possibility that optimal flowering in *Pharbitis* may require a mixture or balance of Pfr and Pr at the 8-hour point.

In most of these early works the plants were subjected to short days consisting of 24-hour cycles. However, when plants are subjected to very long cycles (48–72 hours), they show a rhythmic response to red light interruptions. Recently Carpenter and Hamner (3) reported that Biloxi soybean plants did not show a rhythmic response to far-red interruptions, but did show a clear rhythmic response to red light interruptions. Könitz (5), on the other hand, reported opposite results, i.e., *Chenopodium* plants showed a rhythmic response to far-red interruptions in that the far-red light exerted an effect quite opposite to red light at any point in the photoperiodic cycle.

Thus, many of the results are conflicting and more extensive studies on the effect of far-red light may be required. In the present experiments *Pharbitis* plants were subjected to very long dark periods at suboptimal temperatures and the effect of far-red light and its interaction with red light was studied in detail. The temperature was carefully controlled so that in each experiment the flowering responses would be at the most sensitive level.

Material and Methods

Seedlings of *Pharbitis nil,* strain Violet, were used for all experiments. Experimental methods and procedures were quite similar to those described in a previous paper (10). The far-red light used in the present experiments was obtained from eight 300 w reflector-spot bulbs filtered with Corning filters (No. 2600) and 2 cm of water. Intensity of the far-red radiation at the leaf surface was about 6000 ergs/cm^2 per second (about 4000 ergs/cm^2 per second between 700 and 800 mμ). The red light was obtained from Gro-lux fluorescent lamps filtered with 2 layers of red cellophane, and its intensity at the leaf surface was about 3300 ergs/cm^2 per second. The spectral energy distributions of the red and the far-red lights were measured by an instrument devised for this purpose (2) and are shown in figure 1. The red light has only about 0.3 % contamination of far-red and the far-red light has no measurable red contamination. A small amount (about 1.5 %) of radiant energy in the region of 350 to 400 mμ is involved in the far-red light, but the physiological activity of these wave lengths is unknown.

All plants used in the present experiments were kept under continuous illumination from cool-white fluorescent lamps (400 ft-c) before the experimental treatment. Temperature during the light period was 20° in all experiments.

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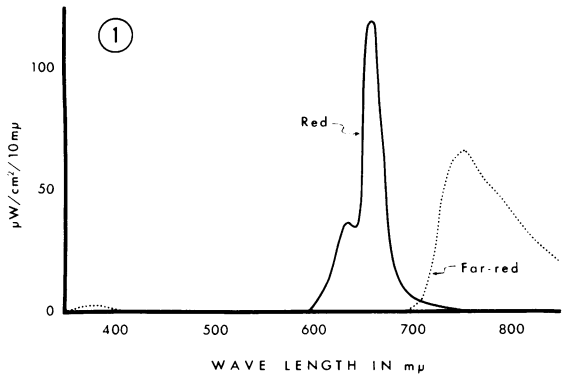

FIG. 1. Spectral energy distribution of red and far-red light sources used in the present experiments. Measured by a spectroradiometer.

Experimental Results

Experiment 1. *Pharbitis* seedlings grown under continuous light for 4 days were placed in darkness for 48 hours at various temperatures and a 5-minute far-red light interruption was given at different times in the dark period (fig 2). At any temperature far-red light applied at the beginning of the dark period was most inhibitory to flowering. The inhibitory effect decreased almost linearly with delay of the far-red irradiation.

Experiment 2. One lot of plants (controls) was subjected to a single dark period of various lengths. Another lot (experimental lot) was also subjected to a single dark period of various lengths but 5 minutes of far-red light was applied at the beginning of each dark period (fig 3). The dark temperature was 20°. The flowering response of the controls increased with increasing duration of the dark period and became saturated when the dark period was longer than 28 hours. Far-red light given at the beginning of the dark period inhibited flowering irrespective of the length of the dark period. It is interesting that if the far-red light was applied at the beginning of the dark period the flowering response did not increase with increased duration of the dark period beyond 16 hours.

In another experiment far-red light was given 8 hours after the onset of darkness followed by various lengths of dark period (fig 4). The flowering response increased with increasing duration of the dark period during the first 18 hours in the same way as that of the control which was not exposed to far-red light. However, the flowering response of the plants exposed to the far-red light did not increase with further increase in the dark period. In other words, far-red light given at 8-hour point did not inhibit flowering if the dark period was shorter than 18 hours, but inhibited flowering if the dark period was longer than 18 hours.

Experiment 3. Three groups of plants were subjected to a 48-hour dark period, and exposed to 5 minutes of red light 4, 8 or 12 hours after the onset of darkness followed by 5 minutes of far-red light applied at different times (fig 5, 6). Red light given at these points inhibited flowering, however, far-red light given shortly after the red interruption was more inhibitory, and the inhibitory effect of the far-red light decreased almost linearly as a function of time after the red exposure. The flower-inhibitory effect of a red interruption was never reversed by subsequent far-red irradiation.

Experiment 4. Plants were subjected to a 48-hour dark period and exposed to 5 minutes of far-red light at the beginning, or 8 hours after the beginning, of the dark period followed by 5-minute red light interruptions applied at different times (fig 7). Another group of plants, which served as controls, was exposed to a single red light interruption of 5 min-

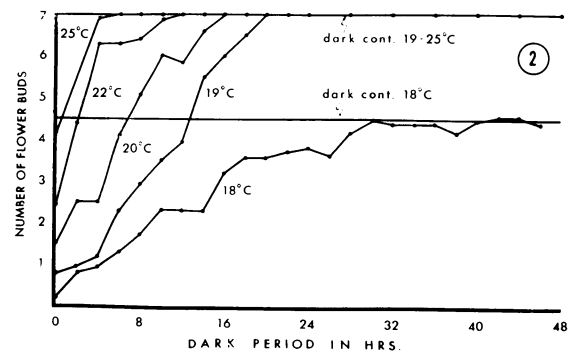

FIG. 2. Flowering response of *Pharbitis* exposed to 5 minutes of far-red light (6000 ergs/cm² per sec) at different times in a 48-hour dark period.

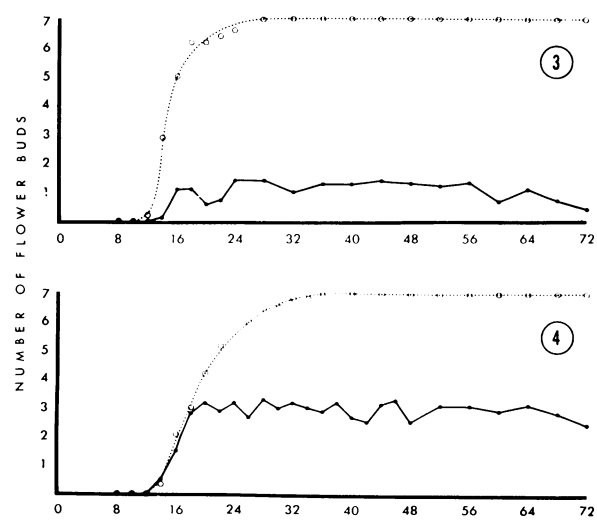

FIG. 3 and 4. Effect of far-red light (5 min) given at the beginning (fig 3) or 8 hours after the beginning (fig 4) of the dark period of which lengths are variable. Dotted line shows the flowering response of control plants which were subjected to various lengths of dark period without receiving far-red light. The dark temperature was 20° in figure 3, and 19° in figure 4.

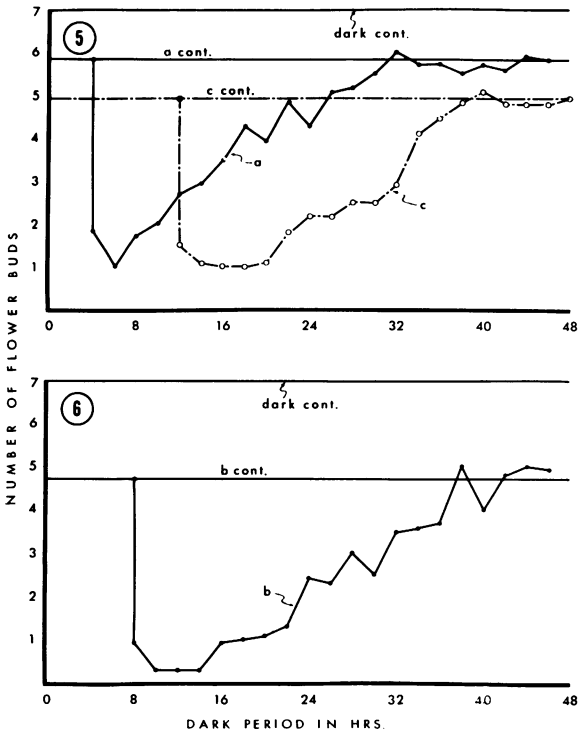

FIG. 5 and 6. Flowering response of *Pharbitis* exposed to 5 minutes of red light followed by 5 minutes of far-red light after different intervals. Curve a: Plants were exposed to 5 minutes of red light 4 hours after the beginning of a 48-hour dark period, and thereafter exposed to 5 minutes of far-red light applied at different times. Curves b and c: Similar to curve a but the red light was given 8 and 12 hours, respectively, after the beginning of the dark period. The dark temperature was 19° in figure 5, and 22° in figure 6. Controls for a, b and c: Flowering response of control plants which were exposed to 5 minutes of red light 4, 8 and 12 hours, respectively, after the beginning of the dark period without any subsequent far-red irradiation.

utes at different times in a 48-hour dark period. Far-red light applied at the beginning of the dark period inhibited flowering strikingly ; however, red light given shortly after the far-red irradiation repromoted a flowering response. Red light applied even 12 to 24 hours after the far-red irradiation repromoted flowering to some extent, however, red light applied at the 8-hour point did not. When far-red light was applied 8 hours after the beginning of the dark period, red light given immediately after the far-red inhibited flowering. However, red light applied between the 12- to 24-hour points repromoted flowering. In both groups to which far-red light was given at the beginning and 8 hours after the beginning of the dark period, the flower-promoting effect of red light decreased with increasing intervals of time between the far-red and red irradiations, and red light applied during the last 20 hours of the dark period inhibited flowering slightly with a maximum between the 32- and 36-hour points. It is interesting that even if far-red light was given at the beginning or 8 hours after the beginning of the dark period, the red light applied during the first 8 hours of the dark period had an effect similar to that obtained when a single red light interruption was given without giving any far-red light.

In another experiment plants were subjected to a 24-hour dark period and exposed to 5 minutes of far-red light at the beginning of the dark period followed by 5-minute red light interruptions applied at different times (fig 8). Red light interruptions given within the first 4 hours restimulated flowering, but those given from the 6- to 12-hour points inhibited flowering with a maximum at the 8-hour point. When the dark period was 48 hours, red light applied at the 12- to the 24-hour points promoted

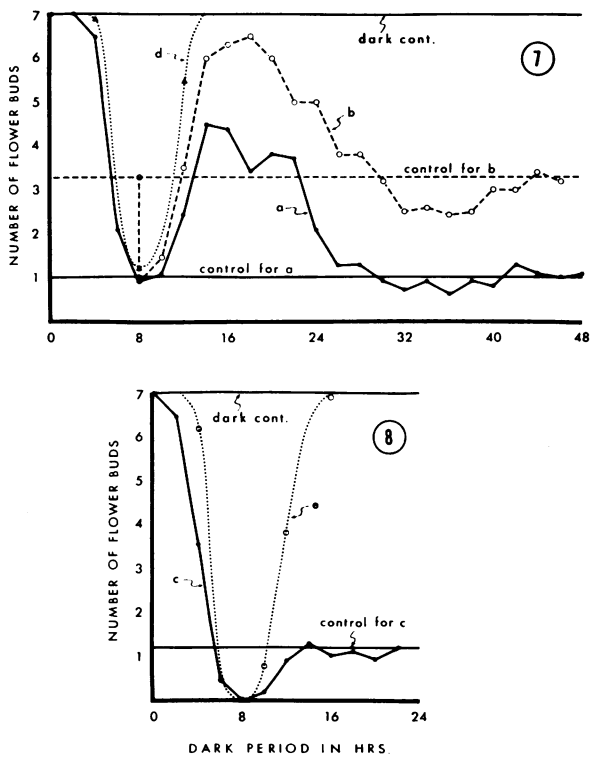

FIG. 7 and 8. Flowering response of *Pharbitis* exposed to 5 minutes of far-red light followed by 5 minutes of red light after different intervals. Curve a: Plants were exposed to 5 minutes of far-red light at the beginning of the dark period, and thereafter exposed to 5 minutes of red light at different times. The dark temperature was 19°. Curve b: Similar to curve a but the far-red light was given 8 hours after the beginning of the dark period. Curve c: Similar to curve a but dark period was 24 hours and the dark temperature was 20°. Curves d and e: Flowering response to a single red light interruption (5 min) applied at different times during 48- and 24-hour dark period, respectively. Controls for a, b and c: Flowering response of control plants which were exposed to 5 minutes of far-red light without any subsequent red irradiation.

flowering (fig 7). However, when the dark period was 24 hours red light given at these points did not promote flowering (fig 8).

Experiment 5. Four groups of plants were subjected to a 48-hour dark period at 20°, and given the following light treatments at different times during the first 16 hours of the dark period (fig 9). Group 1 : 5 minutes of red light (R), Group 2 : 5 minutes of far-red light (FR), Group 3 : 5 minutes of red light immediately followed by 5 minutes of far-red light (R + FR), Group 4 : 5 minutes of far-red light immediately followed by 5 minutes of red light (FR + R). Flowering responses to a single red and a single far-red light interruption were just as expected [cf. fig 2, and previous paper (9)]. Interruptions with far-red followed by red light showed the same effect as that of a single red light interruption. However, interruptions with red followed by far-red light were very inhibitory at the 8- to 10-hour points. The experimental curves shown in figure 9 suggest that the inhibitory effects of red and far-red light interruptions were almost additive when they were given in that order.

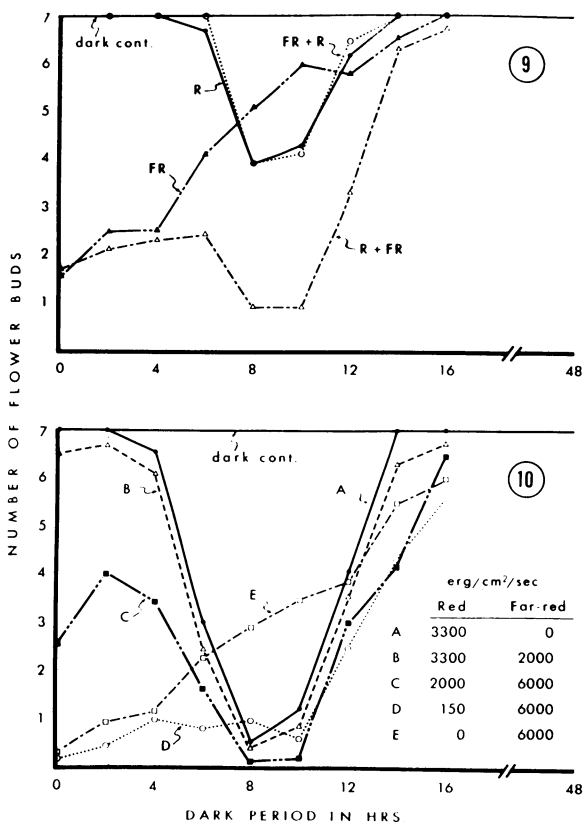

FIG. 9. Flowering response to red and far-red light or either one followed by the other, given at different times during the first 16 hours of a 48-hour dark period. The dark temperature was 20°. R : 5 minutes of red light (3300 ergs/cm² per sec). FR: 5 minutes of far-red light (6000 ergs/cm² per sec). R + FR: 5 minutes of red light immediately followed by 5 minutes of far-red light.

FIG. 10. Flowering response to mixed red and far-red light given at different times during the first 16 hours of a 48-hour dark period. The dark temperature was 19°. Mixed lights shown in figure were given for 5 minutes at different times.

Experiment 6. Five groups of plants were subjected to a 48-hour dark period and exposed to 5 minutes of mixed red and far-red light (fig 10). Energies of red and far-red lights are shown in figure 10. The flowering responses to pure red (Group A) and pure far-red (Group E) are just as expected, and the slight contamination of far-red in the red light hardly changed the red light effect (Group B). However, the small amount of red light contamination in far-red light caused strong inhibition at the 6- to 10-hour points (Group D). When mixed light was given at the beginning of the dark period, the flowering response became more inhibited as the ratio of red light to far-red light decreased. However, at the 8- to 10-hour points flowering responses were related to the total energy of red and far-red rather than the ratio of red to far-red.

Discussion

As has been discussed in previous papers (10, 11), there are at least 3 kinds of timing mechanisms in the photoperiodic response of *Pharbitis*. The first component is similar to an hourglass in that a linear increase in the flowering response results with increasing duration of the dark period. The second component is an endogenous circadian rhythm which starts at the beginning of the light period (the light-on rhythm). The third component is also an endogenous circadian rhythm which starts at the beginning of the dark period (the light-off rhythm). This rhythm has a red sensitive phase with a maximum 8 hours after the onset of darkness. In the present experiments plants were kept under continuous light before the dark period, i.e., there was no light-on signal before the dark period. Therefore, the light-on rhythm was not participating in the flowering response.

The flowering response to far-red interruptions did not show a rhythmic response (fig 2). Far-red light applied at the beginning of the dark period was most inhibitory to flowering, and the inhibitory effect of a far-red interruption decreased almost linearly with delay of far-red exposure. This suggests that the effect of far-red is closely related to the hourglass component of the timing mechanism mentioned above, and not to the endogenous rhythm.

Takimoto and Ikeda (13) reported that in *Pharbitis* far-red light applied 8 hours after the beginning of the dark period has a maximum flower-inhibiting effect. In their work, however, a cellophane filter was used to obtain far-red light, and the far-red light had a considerable amount of red contamination. The far-red light used in the present experiments has no

measurable red contamination. However, when far-red light was mixed with a small amount of red light the flowering responses to interruptions with this mixed light (fig 10) were very similar to those reported by Takimoto and Ikeda. The maximum inhibitory effect of far-red light at the 8-hour point reported by Takimoto and Ikeda was apparently caused by the red light contamination.

If a far-red interruption was followed by various lengths of dark period the flowering response did not increase with increased duration of the dark period beyond certain lengths. On the other hand flower inhibition caused by a red light interruption is overcome, though not completely, by prolonged duration (at least up to 72 hours) of the following dark period (10). It is supposed that the far-red light exerts some effect which completely stops the hourglass component of the timing mechanism after a certain time and the red light, as has been discussed before (11), exerts some effect which slows down the hourglass component.

It has been reported in both *Xanthium* (1) and *Pharbitis* (14) that far-red light applied at the beginning of a short dark period promotes flowering. However, in the present experiments far-red light never promoted flowering even with short dark periods. Recently Fredericq (4) reported that the flower-inhibiting effect of far-red was greater when the light intensity in the main light period was kept rather low, or when the photoperiod was shortened to 2 to 4 hours. In the present experiment the light intensity was kept relatively low. If the light intensity had been kept very high the far-red might have had some flower-promoting effect. However, even if the light intensity was kept very high (sun light), and a long light period was given before the dark period, the far-red light applied at the beginning of the dark period inhibited flowering when the length of the dark period was longer than 13 hours (14). Under these conditions far-red light applied at the beginning of the dark period might have had a slightly promotive effect on the hourglass component of the timing mechanism during the first 13 hours. However, even in this case the hourglass component may have been stopped after a certain duration of time.

The flower-inhibitory effect of red light was not reversed by succeeding far-red irradiation, and far-red light following a red interruption was more inhibitory to flowering than red light alone (fig 5, 6). That is, when far-red follows red light the inhibitory effects of red and far-red light are additive. The inhibitory effect of far-red light decreased with increased intervals of time between the red and far-red light interruptions (fig 5, 6). It is assumed that when red light was given in an inhibitory phase of the light-off rhythm the hourglass component of the timing mechanism was slowed down and resulted in flower inhibition, but the following far-red irradiation exerted another effect which stopped the hourglass component after a given time (see fig 3, 4), and resulted in increased inhibition. If the time of far-red irradiation was delayed some of the hourglass component proceeded, even though it proceeded very slowly, before the far-red irradiation and only the remaining process was affected by far-red. Thus, the flower-inhibiting effect of the far-red decreased with increasing interval between the red and far-red interruptions.

If, however, the far-red irradiation was followed by red, the inhibitory effect of far-red was reversed by the red light provided that the time of red irradiation did not fall in the inhibitory phase of the light-off rhythm (fig 7). Red light applied even 16 to 24 hours after far-red irradiation repromoted the flowering response to some extent, and the repromoting effect of red light decreased with increasing interval between the far-red and red irradiations (fig 7). As has been discussed above, far-red light may have an effect which stops the hourglass component of the timing mechanism and results in flower inhibition. Red light may reverse the far-red effect even if the time between red and far-red irradiations is in excess of 16 hours, and reset the hourglass component. When the time of red irradiation is delayed, however, the length of the following dark period is reduced. Even if the hourglass component is reset by red light a long dark period is required to repromote flowering. Therefore, the repromoting effect of red light is reduced with delayed time of red irradiation. When far-red light was applied at the beginning of a 24-hour dark period (fig 8), red light given at the 16- to 24-hour points did not repromote flowering because the length of the dark period after the red irradiation was too short.

The curves shown in figures 7 and 8 have a big dip at the 8-hour point. The response curve to a single red light interruption also has the same dip at the 8-hour point. This means that the light-off rhythm still persists after far-red irradiation, and red light given during this inhibitory phase results in flower inhibition. In figure 7 the red light applied during the last 20 hours of the dark period slightly inhibited flowering. A similar effect of red light was reported previously (12), and this effect was considered to be based on an interaction with the following light period.

The effect of red light is not reversed by far-red, but the effect of far-red is reversed by red light. This interrelation is clearly shown in figure 9. If the dark period was interrupted with FR + R (far-red immediately followed by red) at different times, the flowering responses were quite similar to those obtained with a single red light interruption. On the other hand, if the dark period was interrupted with R + FR (red immediately followed by far-red) the inhibitory effects of both red and far-red were additive (fig 9). When mixed red and far-red light was applied at the beginning of the dark period the flower-inhibiting effect was greater when the ratio of red to far-red was small (fig 10). However,

when the mixed light was given at the 8- to 10-hour points the flowering responses were not related to the ratio of red to far-red, but rather to the total energy of red and far-red. The flower-inhibiting effect of far-red light applied at the beginning of the dark period may be controlled by phytochrome, because the response is related to the ratio of red to far-red and also the inhibitory effect of far-red is reversed by red light. However, the flower-inhibitory effect of light at the 8-hour point is not related to the red-far-red ratio but to the total amount of red and far-red energies. These phenomena may not be understood with the current general concept of the physiological action of the phytochrome system. This problem will be discussed in another paper.

From the experiments presented here it is supposed that the flower-inhibiting effects of red and far-red are based on different mechanisms. Red light exerts some effect which slows down the subsequent progress of the hourglass component of the timing mechanism, and far-red light exerts some effect which stops the hourglass component after a given duration of time. The far-red effect is reversed by red light, but the red effect is not reversed by far-red light.

Summary

Seedlings of *Pharbitis nil*, strain Violet, were used for all experiments. When a 48-hour dark period was interrupted with 5 minutes of far-red light at different times, the far-red light applied at the beginning of the dark period was most inhibitory to flowering. The inhibitory effect of the far-red light decreased almost linearly with delay of far-red irradiation. When far-red light was given at the beginning or 8 hours after the beginning of the dark period followed by various lengths of dark periods, the flowering response did not increase with increased duration of the dark period beyond certain lengths. These results suggest that far-red light exerts some effect which stops the following dark process required for flowering after a certain duration of time.

Far-red light given shortly after a red interruption was very inhibitory to flowering, and this inhibitory effect decreased with increasing intervals of time between the red and far-red irradiations. However, the red light applied after far-red repromoted flowering provided that the time of the red irradiation did not fall in an inhibitory phase of the light-off rhythm which was initiated by the beginning of the dark period. The flower-repromoting effect of red light decreased with increasing intervals of time after the far-red irradiation, but red light given even 24 hours after the far-red irradiation repromoted flowering to some extent.

If the inductive dark period was interrupted with 5 minutes of red light followed by 5 minutes of far-red light at the 8-hour point (the most red-sensitive phase), the flower-inhibiting effects of red and far-red light were almost additive. However, if the red and far-red light were given in reversed order they showed the same effect as a single red light interruption. The flower-inhibiting effect of far-red light applied at the beginning of the dark period was reduced by simultaneous irradiation with red light (mixed light), but the inhibitory effect of red light at the 8-hour point of the dark period was intensified by simultaneous irradiation with far-red light. Possible roles of far-red light in the photoperiodic response were discussed.

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