Inhibition of Flowering of Xanthium pensylvanicum Wallr.
by Prolonged Irradiation with Far Red

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Summary. Interrupting each long night with a prolonged period of far red radiant energy resulted in the inhibition of floral initiation in cocklebur. Irradiations inducing different relative levels of P_{FR} from 1 to 2 % to 80 % had about the same effect under 4-hour photoperiods. The lower levels of P_{FR} induced by continuous far red irradiation were not as effective as the higher levels induced by red under 8 and 12-hours photoperiods. The critical P_{FR} level required to induce inhibition of flowering seems to increase with increasing photoperiods.

Flowering of many kinds of plants is controlled by red and far red radiant energy which induces a change in the photoreceptive pigment phytochrome. Interruption of a long night with red irradiation causes inhibition of flower initiation in short-day plants (3). A brief far red irradiation following the exposure to red generally repromotes flowering (1), but a prolonged irradiation with far red near the middle of the dark period often inhibits flowering (1, 2, 4, 5). Kasperbauer et al. (5) showed in Chenopodium rubrum that treatments of the same total duration with far red, or different combinations of red, far red, and darkness produced similar inhibitory results. Preliminary experiments with cocklebur, however, indicated a somewhat varied response to prolonged exposures to various combinations of far red, darkness and red radiant energy. The differences in response were therefore studied in some detail and the results were reported here.

Materials and Methods

The cocklebur plants used in these experiments were grown in a greenhouse until the fifth leaf was completely expanded. The plants were grown under natural day plus a 3-hour interruption near the middle of the night from incandescent-filament lamps. The experimental treatments were given in growth rooms to which the plants were moved the day preceding the starting of the treatments. The light sources in the growth rooms were cool white fluorescent and incandescent-filament lamps providing a total illumination of 2000 ft-c at plant level. About 10 % of the 2000 ft-c was provided by the incandescent lamps. Red radiant energy was obtained by filtering the light from a bank of 18 cool white fluorescent lamps through 2 layers of red cellophane. Total energy between 600 and 680 mJ was 600 μW cm⁻². Far red radiant energy was obtained by filtering the light from three, 300 w reflector flood incandescent lamps through 2 layers of blue and 2 layers of red cellophane and 5 cm of water. Total energy between 710 and 800 mJ was 750 μW cm⁻². Sources of equal spectral composition, but of lower energy were tested for the P_{FR} level induced at the photostationary state in phytochrome solutions and in Avena seedlings. The red source gave a 76 to 78 % P_{FR} in solution and a 75 to 79 % P_{FR} in Avena seedlings at the photostationary point. The far red source gave 1.5 to 2.5 % P_{FR} in phytochrome solution and 1 to 3 % P_{FR} in Avena seedlings at the photostationary point.

Experimental treatments were given for 3 cycles and then the plants were returned to the greenhouse under a noninductive photoperiod until dissection for the determination of the flowering stage of the terminal bud. All plants were dissected 10 days after the start of the treatment. The flowering stage of the terminal bud was determined on the basis of a scale of values from 0, vegetative, to 7 (1). Each experimental treatment was repeated at least 3 times, each time on a lot of 6 plants.

The prolonged irradiations with different combinations of red, far red, and darkness were as follows: given X = total duration (in minutes) of the treatments, the 4 different treatments were: A) continuous far red for X minutes; B) 4 minutes red, then (X-4) minutes far red; C) (X-8)

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Fig. 1. Action of different combinations of red and far red applied around the middle of different long nights on flowering of cocklebur. A = 40 min FR; B = 4 min R - 36 min FR; C = 32 min R - 8 min FR; D = 4 min R - 28 min dark - 8 min FR. Flowering stages are expressed as percent of the flowering of the controls on uninterrupted nights. Average flowering stages (12 plants) for dark controls were 6.9, 6.7, 6.8 for the 12-, 16-, and 20-hour night respectively.

Fig. 2. Action of different combinations of red (R), far red (FR), and darkness (D), applied at different times during long nights, on the flowering of cocklebur. Flowering stages are expressed as percent of the flowering of the controls on uninterrupted nights. Fig. 2a = 12-hr night; fig. 2b = 16-hr night; fig. 2c = 20-hr night. •——• = 60 min FR; ▲-▲ = 4 min R - 56 min FR; ■■■ = 52 min R - 8 min FR; ○——○ = 4 min R - 48 min D - 8 min FR. Average flowering stages (18 plants) for dark controls were 6.8, 6.6, and 6.8 for the 12-, 16-, and 20-hour night respectively.
Table I. Action of Red (R) and Red Plus Far Red (FR) Applied in the Middle of Nights of Different Lengths on Flowering of Cocklebur

Flowering stages are expressed as % of the controls on uninterrupted nights.

<table>
<thead>
<tr>
<th>Night length (hrs)</th>
<th>Flowering stage (%)</th>
<th>4 min R</th>
<th>4 min R-4 min FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 (6.6-24-0.78)*</td>
<td>0</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>16 (6.8-24-0.36)*</td>
<td>0</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>20 (6.8-18-0.37)*</td>
<td>0</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>22 (4.1-24-1.10)*</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parenthesis indicate average flowering stage, number of plants and standard deviation of the dark controls.

minutes red, then 8 minutes far red; D) 4 minutes red, then (X-12) minutes dark, then 8 minutes far red.

The relative P_{FR} level at the end of each 1 of the 4 treatments would be 1 to 2%.

Results

An interruption near the middle of a long night with red radiant energy results in the inhibition of flowering. Far red applied immediately after red reverses the inhibition (table I), the degree of repromotion becoming less as the night becomes longer (7).

A prolonged period of irradiation with far red applied near the middle of the night results in the inhibition of flowering (table II). The degree of inhibition depends on the duration of the photoperiod (table II).

In Chenopodiurn rubrum, treatments of the type A-B-C-D, outlined above, would all cause the same amount of inhibition of flowering (5). In cocklebur the amount of inhibition produced by similar treatments is not the same. Treatments C and D are more effective than treatments A and B. The difference between the degree of inhibition induced by the various irradiations decreases with an increase in the night length (fig 1).

Table II. Action of Prolonged Far Red (FR) Irradiations Applied Near the Middle of Night on the Flowering of Cocklebur

Flowering stages are expressed as % of the flowering of the controls (DC) on uninterrupted nights.

<table>
<thead>
<tr>
<th>FR treatments (min.)</th>
<th>12 hr</th>
<th>16 hr</th>
<th>20 hr</th>
<th>22 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (DC)</td>
<td>100 (6.7)*</td>
<td>100 (6.8)*</td>
<td>100 (6.7)*</td>
<td>100 (4.2)*</td>
</tr>
<tr>
<td>15</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>85</td>
<td>84</td>
<td>76</td>
<td>38</td>
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<tr>
<td>60</td>
<td>73</td>
<td>45</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>90</td>
<td>61</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>57</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>63</td>
<td>0</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parenthesis indicate average flowering stage of the dark controls.

Since the degree of inhibition could be a function of the numbers of hours of darkness preceding or following the interruption, irradiations of the same total duration, 60 minutes, were given at different times during 12, 16 or 20-hour dark periods. Increasing the duration of the night reduced the differences in inhibition of flowering by the various treatments and increased the efficiency of treatments A and B irrespective of time of irradiation (fig 2a, b, c).

Discussion

Irradiation schedules with red and far red shown in figure 3 should maintain the relative levels of P_{FR} indicated. A saturation dosage of red radiant energy would produce about 80% P_{FR} and a saturation dosage of far red would result in 1 to 2% P_{FR}.

It has been shown (5) that the same level of inhibition of flowering in C. rubrum was induced by any combination of red and far red irradiation.
that maintained $P_{FR}$ at or above a critical level of 1 to 2% when the total durations of the treatments were the same. A level of 80% $P_{FR}$ or a level of 1 to 2% would induce the same response.

In cocklebur, however, inhibitory effectiveness is increased with higher levels of $P_{FR}$ except when nights are very long, i.e., 20 hours or more. One might suppose that inhibition of flowering would be most easily accomplished on shorter nights, 16 and 12 hours, yet the converse was true. Sixty minutes of far red applied near the middle of the night failed to inhibit flowering until the length of the night was increased to 20 hours (table II, fig 2a, b, c). Since the induction of flower primordia in untreated controls was the same on the 3 different photoperiods, one cannot explain the increased inhibitory effectiveness of far red as due to a weaker induction under the 4-hour photoperiod and in fig 1 it is shown that treatment D is equally effective on all 3 photoperiods.

From our results it seems that the relative level of $P_{FR}$ may be the limiting factor under the 12- and 16-hour night, but not under the 20-hour night.

The meaning of these results is not clear, but possible explanations, depending on either de novo synthesis of phytochrome or on differences in levels of substrate, are considered. Recent evidence has been advanced by Spruit (6) for a de novo synthesis of phytochrome as $P_R$ and a photo-bleaching of $P_{FR}$. Under longer photoperiods, more photo-bleaching may occur than resynthesis; this would result in a lower total amount of phytochrome under the 12- and 8-hour photoperiods than under the 4-hour photoperiod. Thus, a relative level of 1 to 2% $P_{FR}$ as induced by continuous far red irradiation might be below the absolute critical level under shorter nights and above it on the 20-hour night. On shorter photoperiods of 4 hours, with less photo-bleaching, 1 to 2% of the higher total amount of phytochrome as $P_{FR}$ might be adequate for a total inhibition of flowering. On longer photoperiods, only the higher relative levels of $P_{FR}$ induced by red radiation would be above the absolute critical level necessary for the inhibition, whereas the low level of $P_{FR}$ maintained by the far red irradiation, being below the absolute critical level, would be much less effective.

Another hypothesis that could be considered is that of different levels of substrate for the $P_{FR}$ controlled reaction under different photoperiodic conditions. Depending on the substrate available, the control of the reaction could be shifted from a condition where $P_{FR}$ is the limiting factor, on shorter nights, to a condition where the substrate is the limiting factor, on longer nights. The final response could be due to the interplay of both factors, level of substrate and total phytochrome.

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**Literature Cited**