Reversal of the Effects of a Night Interruption in *Lemna* by Inhibitors of Ribonucleic Acid Synthesis¹, ²

Received for publication May 23, 1974 and in revised form August 12, 1974

ROBERT P. DOSS³

Department of Environmental Horticulture, University of California, Davis, California 95616

ABSTRACT

The inhibition of flowering of *Lemna perpusilla* Torr. strain 6746 caused by a light break can be partially reversed by treatment with actinomycin D or 2-thiouracil. Actinomycin D is most active in reversing the response to a light break if the inhibitor is present in the fronds at the time the light break is administered.

A light break given during an inductive dark period can inhibit floral induction in the short day plant *Lemna perpusilla* 6746 (3, 4). The mechanism whereby a light break inhibits flowering in short day plants is not clear. However, because red-far red reversibility is often observed, phytochrome participation in the light break response is indicated (4, 6). Inhibitors of RNA synthesis can influence phytochrome controlled behavior in some plants (8). In results reported here, it is shown that two inhibitors of RNA synthesis, actinomycin D and 2-thiouracil, can partially reverse the inhibition of flowering caused by a light break in *L. perpusilla* 6746.

MATERIALS AND METHODS

Stock culture of *Lemna perpusilla* Torr strain 6746 were grown in 125-ml Erlenmeyer flasks containing 50 ml of half-strength Hutner's medium supplemented with 1% (w/v) sucrose. Experimental cultures were started with single, three frond colonies using 10 ml of the stock culture medium contained in plastic capped 25 × 100 mm test tubes. All cultures were grown axenically at an air temperature of 26 ± 1°C under cool white fluorescent light (250–350 ft-c). Stock cultures were grown under continuous light.

Experimental cultures were maintained for a total of 7 days. On the first and last 2 days of the experimental period, the plants were grown under continuous light. On the 3rd, 4th, and 5th days, the plants were exposed to inductive photoperiodic cycles consisting of 8 hr of light and 16 hr of darkness (1).

At the 13th hr of the first inductive photoperiodic cycle, plants were transferred into 2 ml aliquots of half-strength Hutner's medium supplemented with 1% (w/v) sucrose or into identical medium containing either 78 μM 2 thiouracil or 0.8 μM actinomycin D (Calbiochem). Inhibitor solutions were prepared on the day of use and sterile-filtered. At the 15th hr of the first inductive photoperiodic cycle, the plants were removed from the transfer solutions and returned to the experimental culture vessels.

Plants were exposed to a 2-min warm white fluorescent light pulse (100–150 ft-c) ending at the 14th hr of the photoperiodic cycle (Fig. 1) or to similar light pulses ending at the 13th, 14th, 15th, and 16th hr (Fig. 2). All operations during the inductive dark period were performed using a dim green safelight.

Per cent flowering and total fronds were determined as described by Hillman (3). Data were analyzed by analysis of variance after subjecting the per cent flowering values to an angular transformation or by Student's *t* test (7).

RESULTS AND DISCUSSION

Figure 1 shows that actinomycin D and 2-thiouracil can reverse the inhibition of flowering brought about by a light

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5H+S</th>
<th>ACT-D</th>
<th>2-TU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent flowering</td>
<td>67.0%</td>
<td>85.0%</td>
<td>65.0%</td>
</tr>
<tr>
<td>Fronds</td>
<td>69.0°C</td>
<td>64.38</td>
<td>59.7B</td>
</tr>
</tbody>
</table>

Fig. 1. Influence of actinomycin D and 2-thiouracil on the response of *L. perpusilla* 6746 to a light break. See text for details of experiment. Shaded bars represent average per cent flowering of cultures exposed to a light break; open bars represent average per cent flowering of cultures not exposed to a light breaking. Numbers over the bars indicate average total fronds for each set of three replicates used per treatment. Bars bearing different superscripts represent significantly (*P* = 0.5) different per cent flowering values. ACT-D: actinomycin D; 2-TU: 2-thiouracil; 0.5 H + S: half-strength Hutner’s medium supplemented with 1% (w/v) sucrose.

¹ This research was supported in part by a Chancellor’s Patent Fund Grant and a Priority C Research Grant, University of California, Davis.

² A portion of this report is taken from a dissertation submitted in partial fulfillment of the requirements for a Ph.D. degree in Plant Physiology at the University of California, Davis.

³ National Defense Education Act predoctoral fellow.
Fig. 2. Influence of actinomycin D and light breaks on the flowering of *L. perpusilla* 6746. See text for details of experiment. Closed symbols are used to show the response to 2-min light breaks given while plants are in the transfer solution. Open symbols are used to show the response to light breaks given while plants are in experimental culture vessels. Actinomycin D: □ and ■; half-strength Hutner's solution supplemented with 1% (w/v) sucrose: ◆ and ◆. Each datum point represents the mean for three replicates. Points on the y-axis represent the response without light breaks. Asterisks indicate significant (P = 0.05) difference in percent flowering between actinomycin D treated and control cultures which receive a light break at the same time.

break. Little influence on vegetative growth is seen nor does inhibitor treatment significantly influence the percent flowering of cultures not exposed to a light pulse. Actinomycin D is most effective in reversing the inhibition of flowering caused by a light break if inhibitor treatment coincides with the light break (Fig. 2). If the light break is given 1 hr after transfer of the plants from the actinomycin D solution, inhibition of flowering is nearly as great as that seen for the controls.

Actinomycin D and 2-thiouracil are reported to inhibit gene transcription (5) and RNA synthesis (2), respectively. Phytochrome has been shown to be involved in the light break response of *L. perpusilla* 6746 (4), and Mohr (8) has shown that some phytochrome responses in *Sinapis alba* seedlings can be inhibited by treatment with actinomycin D. He suggests that gene transcription, initiated by the appearance of the far red-absorbing form of phytochrome, is inhibited by actinomycin D. Thus, a light break may prevent flowering by activating genes promoting vegetative growth.

Alternatively, a light break may act by rephasing the time-measuring system of the plant so that light interacts with a sensitive phase of a circadian oscillation (9). If this is the case, actinomycin D and 2-thiouracil may prevent the light-induced rephasing.

Acknowledgment—Thanks are due to Dr. Roy M. Sachs for help in preparing the manuscript.

LITERATURE CITED