Participation of Long-Day Inhibition in Flowering of Xanthium strumarium L.  

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ABSTRACT

Two basic experiments defined a long-day inhibitory effect on Xanthium flowering: the basal half of a single leaf on long day inhibits response of the tip half to a short day; and a long-day leaf inhibits response of a short-day leaf, providing it is between the short-day leaf and a receptive bud (whether above or below the short-day leaf). Five hypotheses were explored with the conclusions that the tip half can synthesize florigen, and inhibition is not due to prevention of florigen synthesis, translocational effects, or a translocatable long-day inhibitor. Inhibition is localized and may be a condition of the leaf or a relatively immobile substance. Studies of critical dark period, light intensity, and interruption of a dark period show that, when the leaf is not producing florigen, it is actively inhibitory. Immature leaves are more inhibitory than older leaves. The effect was found not to pass dead tissue, and iron-deficient tissue will cause inhibition, though it will not cause promotion.

Xanthium strumarium (cocklebur) has been widely used in flowering research, but seldom have inhibitor studies used this plant. Perhaps this is because evidence for a promotive florigen is so strong in this plant (23), and inhibitor studies have traditionally used plants requiring several inductive cycles (18). Hamner and Bonner in 1938 (14) indicated that fully expanded leaves under long-day conditions on receptor branches of two-branched plants were inhibitory to floral evocation* of a donor branch subjected to an inductive photoperiod. In 1956, Lincoln, Raven, and Hamner (19) used the same donor-receptor system, finding that inhibition could be quantitatively related to the amount of mature leaf area exposed to long-day conditions, carbohydrate movement toward the receptor branch was necessary for its evocation, and immature leaves were capable of inhibition. Days incorporating 7 1/4 hr of darkness or less were inhibitory to their receptor branches, and only a part of their data could be explained on the basis of assimilate translocation.

The processes of induction and evocation constitute a system of many interrelated factors that must be considered as a whole. Could the lack of success in extraction and identification of flower-promoting factors be caused by ignoring flower-inhibiting factors? If we apply an extract from a flowering plant to one under noninductive conditions, the promotive factor in the extract could be nullified by inhibitors in the vegetative plant acting upon it or the site of its action.

There are several reports of floral-inhibiting factors besides those summarized above relating to cocklebur. Schwabe (24) studied the inhibitory effect of long days interspersed among short days. In flowering of Biloxy soybean, 1 long day nullified the following 2.2 short days. Similar experiments have been done on Kalanchöe by Harder and Bünsow (15) and Schwabe (24). Fratianne (10) demonstrated the likelihood that an inhibitory substance or substances, produced in the leaves of various short-day plants, inhibits flowering in dodder (Cuscuta campestris). Flowering experiments with Fragaria (11–13, 27) strongly demonstrate the participation of inhibitor(s). Promotive factors may not even be necessary to explain induction of this plant. Evans (5–7, 9) has demonstrated a photoperiodic floral inhibitor in the long-day plant Lolium temulentum. Apparently the inhibitor is transported to the bud, where it acts. Evidence with “C-labeled assimilates disputes the hypothesis that translocational and dilutional effects cause inhibition (9). He suggested that abscisic acid may be the short-day inhibitor in Lolium (7).

Chalikyan in 1945 (3) induced various parts of a single leaf of Perilla, while other parts remained under noninductive conditions. The basal leaf half could strongly induce flowering under short days, even when the tip half was under long days, but the reverse was not true. Harder, Westphal, and Behrens (16) obtained similar results with Kalanchöe. In addition, they showed that flowering resulted when the apical half was induced, providing basal leaf tissue was trimmed off.

In related experiments Lona (20) demonstrated that induction of the apical half of the Perilla leaf did not render the basal part effective as a donor in grafting experiments. The basal and apical halves could exist side by side in the leaf without affecting one another.

Inhibition may also be the result of interference with translocation of florigen to the bud. Chalikyan and Butenko (4) correlated movement of “C-labeled assimilates and flowering of Perilla. Conditions promoting transport of assimilates from induced leaves to the buds also promoted flowering; conditions that blocked this translocation were inhibitory.

In the present study, two experiments clearly indicate a long-day inhibitory effect in flowering of Xanthium. Five hypotheses were devised to account for this effect. Experiments were then designed to test these hypotheses, essentially elimi-
nating all but one. Further experiments were performed to study the properties of this long-day inhibition.

**MATERIALS AND METHODS**

Culture methods have been described (22). To summarize, plants of *Xanthium strumarium* L. (cocklebur) were germinated in sand, transplanted into 4-inch square plastic pots after about 2 weeks, and kept vegetative by extending day length to 20 hr with fluorescent light. The length of the night was kept constant regardless of natural day length by illuminating from 2:00 A.M. to 10:00 A.M. and again from 4:00 P.M. to 10:00 P.M.

Eight-week-old plants were usually used. The smallest leaf longer than 1 cm (midrib) was called leaf 1, the next largest, leaf 2, and so on. Plants were used with the no. 3 leaves (maximally sensitive to induction [22]) between 6.9 and 8.5 cm long. Typically, all leaves except leaf 3 were removed the day before induction, but other leaves were sometimes used, as noted. Leaf 3 was used as the source of promotion in all experiments. The tip half of the leaf was usually covered with an envelope of black construction paper for 16 hr, while the basal half was exposed to various light and dark treatments. When two leaves were used, the entire no. 3 leaf was covered, while the entire no. 2 (or other) leaf was treated in various ways. Flowering was scored after 9 days according to a system of floral stages (22). The temperature during treatment was 23°C, unless stated otherwise.

Plants used in the iron deficiency experiment (no. 11) were transplanted to pots containing washed perlite, and the pots were placed in plastic-coated steel trays containing Hoagland's solution minus iron to a depth of 1/2 inches. After marked iron deficiency symptoms appeared, iron chelates corrected the chlorosis within 24 hr. Because iron moves very slowly except in the xylem, it was possible to correct the deficiency in one or more leaves by foliar application of iron chelite without correcting the deficiency in other parts of the plant. The leaves were dipped in a solution of 4 g/liter iron chelate [Geigy Sequestrene 138 Fe, active ingredient: technical sodium ferric ethylenediamine di-(o-hydroxyphenylacetate)], and the excess was blotted off.

**RESULTS AND DISCUSSION**

In experiment 1, basal or tip halves of leaf 3 were covered for 16 hr, normally an optimal inductive dark period. Unshaded areas were kept in continuous incandescent light of about 15 ft-c intensity. Plants with the basal halves covered flowered only slightly less than controls with entire leaves covered (Fig. 1), but plants with the tip half covered were completely vegetative.

Experiment 2 tested the ability of leaf 2 to inhibit flowering when leaf 3 was induced. Leaf 2 was removed from controls. Leaf 3 was induced, but leaf 2 received 10 ft-c of continuous incandescent light. Leaf 2 completely inhibited the action of the induced leaf 3. Hence we confirm that immature leaves are capable of strongly inhibiting the photoperiodic stimulus (19).

We were able to propose the following five hypotheses to account for the results of experiments 1 and 2.

1. The tip half of the leaf is not capable of induction (experiment 1).
2. An inhibitor is produced on long days that inhibits production of promotory by short days (as in Schwabe's experiments [24]).
3. The apparent inhibitory effects depend upon florigen moving only with the assimilate stream. For example, long-day tissue may be acting as an assimilate source (photosyn-thesizing) in such a manner that assimilate from the short-day tissue cannot reach the bud. Alternatively, under some conditions (low light intensity), the long-day tissue may be acting as a sink for assimilate produced by the short-day tissue.
4. There is a long-day-produced, translocatable inhibitor.
5. A substance or condition inhibitory to flowering is produced on long days, and its effect is localized near the tissues in which it originates.

Experiment 3 was designed to test the first two hypotheses. In all cases the tip half of leaf 3 was covered for 16 hr. In treatment 3, tissue was removed from the basal half (leaving no leaf tissue on the veins) before covering the tip half; in treatment 4, basal tissue was exposed to 2-ft-c incandescent light during induction of the tip and then removed. The first two treatments (Fig. 3) demonstrate the same promotion and inhibition as in experiment 1, but flowering occurred in treatments 3 and 4, where the leaf tissue of the basal half of the leaves was removed. The tip half of the cocklebur leaf is therefore fully capable of induction, eliminating the first hypothesis. If inhibition were due to some action of an inhibitor on the subsequent production of a promotive factor, plants in treatment 4 would not have flowered because the long-day tissue was removed after the tip half of the leaf had been induced. This eliminates the second hypothesis. (Note that controls in this experiment flowered considerably less than in some other experiments. This is correlated with time of year—lowest in winter—and is familiar from previous work.)

The question of assimilate translocation was studied in several ways. Experiment 3 has bearing on the hypothesis. If the long-day tissue (the base of the leaf) were acting as an assimilate source and the bud and the short-day tissue as sinks, then the long-day tissue would have to be actively photosynthesizing. Yet the long-day inhibition is almost complete with only 2 ft-c of incandescent light, certainly far below the compensation point.

What is the threshold light intensity of the inhibitory response compared to the threshold intensity for promotion?

**Treatment**

<table>
<thead>
<tr>
<th></th>
<th>Av. Floral Stage</th>
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<tbody>
<tr>
<td>Control, #3 leaf 16 hr. dark</td>
<td>6.00±0.45</td>
</tr>
<tr>
<td>Basal 1/2 #3 leaf 16 hr. dark, tip 1/2 in continuous light</td>
<td>5.20±0.83</td>
</tr>
<tr>
<td>Basal 1/2 #3 leaf in continuous light, tip 1/2</td>
<td>0.00</td>
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**FIG. 1.** Effect on flowering of dark periods given to the tip or basal half of the leaf. Confidence interval was computed at the α = 0.05 level.

**Treatment**

<table>
<thead>
<tr>
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<th>Av. Floral Stage</th>
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<tbody>
<tr>
<td>Control, #3 leaf 16 hr. dark</td>
<td>5.10±0.51</td>
</tr>
<tr>
<td>#3 leaf 16 hr. dark, #2 leaf 10 ft-c continuous light</td>
<td>0.00</td>
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**FIG. 2.** Inhibitory effects on flowering of leaf 2 on long day.
A preliminary experiment (no. 4) was conducted to determine if the act of holding the leaves stationary would affect flowering. Leaves were held with cardboard and wire braces in three positions: horizontal, vertical, and 45° off vertical. These positions:

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Av. Floral Stage</th>
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<tbody>
<tr>
<td>1</td>
<td>Control, 1 leaf, 16 hr. dark</td>
<td>3.60 ± 1.18</td>
</tr>
<tr>
<td>2</td>
<td>Basal 1/2, 2 hr continuous light, tip 1/2, 16 hr. dark</td>
<td>0.10 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>Basal 1/2 tissue removed before dark period, tip 1/2, 16 hr. dark</td>
<td>2.30 ± 1.55</td>
</tr>
<tr>
<td>4</td>
<td>Basal 1/2, 2 ft-c light than its tissue removed after dark period, tip 1/2, 16 hr. dark</td>
<td>2.20 ± 1.00</td>
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</table>

**FIG. 3.** Effect of removal of the basal long-day leaf tissue on response of tip tissue to short day.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Av. Floral Stage</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Unfixed control</td>
<td>3.90 ± 1.43</td>
</tr>
<tr>
<td>2</td>
<td>Fixed at horizontal</td>
<td>3.60 ± 1.62</td>
</tr>
<tr>
<td>3</td>
<td>Fixed at vertical</td>
<td>3.80 ± 1.06</td>
</tr>
<tr>
<td>4</td>
<td>Fixed at 45° of vertical</td>
<td>3.70 ± 1.22</td>
</tr>
</tbody>
</table>

**FIG. 4.** The effect on flowering of holding the leaves stationary.

**FIG. 5.** The intensity of light needed for long-day inhibition compared to that required for inhibition of short-day promotion. The upper curve resulted from exposing the basal half of the leaf to the light source (tip half shaded). The lower curve resulted from exposing the entire leaf.

were all given an inductive 16-hr dark period along with controls in which the leaves remained free. The results (Fig. 4) indicate no significant difference between treatments. Bunning and Moser (1) reported that the act of holding the leaves stationary and interfering with leaf movements had an adverse effect on the flowering of *Perilla* and *Chenopodium*, but we failed to observe this with *Xanthium*, which also has a distinct diurnal leaf movement.

In experiment 5, a 7½-w incandescent light source was used. This was dimmed to a 75% setting on a powerstat (changing the spectral distribution of the light slightly). The plants were placed in semicircles around the light source at increasing distances, resulting in decreasing intensities of light. The leaf of each plant was held in place with its surface normal to the light rays. Half of the plants had the tip halves of their leaves covered; others were left uncovered. Results are shown in Figure 5. The intensity of light needed for the long-day inhibition (basal halves) is only slightly higher than that required for inhibition of short-day promotion. It is difficult to imagine how an assimilate hypothesis could account for this, making the third hypothesis at least doubtful.

In experiments 6 and 7, the inhibitory effect of mature leaves on long days was investigated: In experiment 6, treatment 1, only leaf 3 remained; in treatment 2, leaves 3 and 4 remained, and in treatment 3, leaves 3, 4, and 5 remained. Leaf 3 was always given a 16-hr dark period, and the other leaves, when present, were kept in 10-ft-c incandescent light.

In experiment 7, all buds including the terminal bud were removed except one located below leaf 4 (treatments 1a and 1b) or leaf 6 (treatments 2a and 2b). Treatments are shown in Figure 7. Treatments 1a and 1b were separated in time from treatments 2a and 2b by a period of 2 weeks, explaining the difference in the flowering level between controls 1a and 2a. The lone bud remaining on the plants was allowed to become active before leaf 3 was induced by giving it a 16-hr dark period, and all other leaves were kept in 10-ft-c continuous light. Compare the relative amount of inhibition in experiments 6 and 7 (Figs. 6 and 7). It is clear from a comparison of these two experiments, as has been shown earlier by various workers (e.g., 14, 16, 25), that to be effective, long-day tissue must be situated between the short-day tissue and the bud, a fact difficult to reconcile with the fourth hypothesis. It is also interesting that the level of inhibition caused by the mature leaves in experiment 7 was low compared to that caused by the immature leaf 2 in experiment 2.

Lincoln et al. (19) reported that the critical day for inhibition was longer than that for promotion (critical night for removal of inhibition being 7½ hr). Hence we studied timing effects in the next two experiments.

Experiment 8 was designed to determine the critical night for removal of inhibition of the leaf base. The tip halves of the leaves were covered for 16 hr, and basal halves were covered with separate envelopes. The basal covers were removed periodically throughout the 16-hr dark period, exposing the basal halves to various dark periods (0–16 hr) followed by 3000 ft-c of light from VHO fluorescent lamps and 200-w incandescent bulbs. Figure 8 shows that the critical day for inhibition under our conditions is the same as that for promotion.

In experiment 9, the entire leaf was given a 16-hr dark treatment, interrupted only on the basal half with 5 min of incandescent light at 50 ft-c. These light breaks were given to various groups of plants at 2-hr intervals throughout the dark period (Fig. 9). The shape of the resulting curve is virtually identical with curves produced by illumination of the entire leaf, although inhibition at 8 hr would then be complete. Apparently, a light interruption of a long dark period (normally promotive) causes the leaf to become actively inhibitory, in
LONG-DAY INHIBITION IN XANTHIUM FLOWERING

<table>
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<tr>
<th>No.</th>
<th>Treatment</th>
<th>Av. Floral Stage</th>
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<tbody>
<tr>
<td>1</td>
<td>Control, #3 leaf 16 hr. dark</td>
<td>5.33±0.38</td>
</tr>
<tr>
<td>2</td>
<td>#3 leaf 16 hr. dark, #4 leaf in light</td>
<td>4.70±0.48</td>
</tr>
<tr>
<td>3</td>
<td>#3 leaf 16 hr. dark, #4 &amp; #5 leaves in light</td>
<td>4.80±0.30</td>
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Fig. 6. Effect of flowering of long-day leaves situated below short-day promotive leaf.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Av. Floral Stage</th>
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<tbody>
<tr>
<td>1a</td>
<td>Control, all buds removed except one at node of #5 leaf, #3 leaf 16 hr. dark</td>
<td>5.70±0.82</td>
</tr>
<tr>
<td>1b</td>
<td>Same as 1a control with #4 leaf remaining and in light</td>
<td>2.75±0.98</td>
</tr>
<tr>
<td>2a</td>
<td>Control, all buds removed except one at node of #7 leaf, #3 leaf 16 hr. dark</td>
<td>6.50±1.35</td>
</tr>
<tr>
<td>2b</td>
<td>Same as 2a control with #6 leaf remaining and in light</td>
<td>3.55±1.39</td>
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</table>

Fig. 7. Inhibition of long-day leaves when situated between the promotive leaf and the flowering bud.

Fig. 8. The critical night for removal of inhibition. The curve resulted from giving various dark periods to the basal half of the leaf while giving the tip 16 hr of dark.

Fig. 9. Inhibition of a 5-min, 50-ft-c light break given at various times during a 16-hr dark period to the basal half of the leaf. The 16-hr dark period on the tip half was uninterrupted.

this case to the products of the leaf tip tissue held in continuous darkness. Indeed, in all of our kinetic experiments, it appears that when conditions do not allow promotion, they lead to an active inhibition. Van Senden (25) in related work conducted experiments in which leaves situated between induced leaves and the bud were made inhibitory by light interruptions in the middle of their respective dark periods.

Experiment 10 was designed to test the ability of the inhibitor to move through dead tissue. Controls consisted of plants with a single induced leaf (no. 3) and plants having both the
induced leaf 3 and the long-day leaf 2 intact. In test plants, leaf 3 was induced, but a section of the petiole of leaf 2 was previously killed with a small jet of flame. In Figure 10, the expected long-day inhibition is clear cut (compare treatments 1 and 3), but inhibition brought about by the leaf with the burned petiole is only slight (probably insignificant). It is important to note that that most of the no. 2 leaves with burned petioles appeared healthy and turgid for several days (75% were turgid after 10 days).

Experiment 11 was designed to study the effect of iron deficiency on inhibition. It was known that iron-deficient plants could not be induced to flower, or that they flowered abnormally even though they were placed in normal nutrient solutions immediately following the photoinductive period (26). This result was apparently not due to low levels of carbohydrate, since applied sucrose failed to overcome the inhibitory effects of iron deficiency. Figure 11 shows the pattern of application and the results. Controls were soil-grown plants of the same age. Iron-deficient plants did not flower, but foliar applications of iron only to the induced leaf reversed this deficiency inhibition. Apparently iron deficiency affects only induction and not evocation. Furthermore, the no. 2 leaves were capable of long-day inhibition whether or not they were deficient in iron.

Experiment 12 was an attempt to learn whether the inhibitor moves or is stationary in the leaf. In all plants, the leaf tissue was cut except for the large veins to separate the basal tissue from the tip tissue. This was done as a matter of course in the experiments of Harder et al. (16). The tip half was then given a 16-hr inductive treatment, and the basal half was exposed to 15-ft-c incandescent light. All plants remained vegetative. Two possible explanations occurred to us. First, the promoter moved through the veins where it partitioned back into the basal tissue, leading to inhibition. Second, inhibitory substances did move at least as far as the veins to destroy or inactivate the promoter.

**DISCUSSION AND CONCLUSIONS**

The first two experiments defined an inhibitory effect in flowering of Xanthium and led to five hypotheses. These, presented above, may be summarized briefly as follows:

1. The tip half of the leaf cannot synthesize florigen.
2. A long-day inhibitor prevents subsequent florigen synthesis.
3. Long-day inhibition results from translocation effects.
4. There is a translocatable long-day inhibitor.
5. Long-day inhibition is localized and hence may be a condition or a relatively immobile substance.

Experiment 3 (removal of basal leaf tissue) clearly eliminates the first hypothesis. It is also apparent from this and other experiments that the inhibitor is not acting upon subsequent florigen synthesis, eliminating the second hypothesis. Several experiments have bearing on the translocation hypothesis. Very low light intensities (well below the photosynthetic compensation point) are sufficient for the long-day inhibition, making the translocation hypothesis suspect. Nevertheless, we plan to study translocation effects more directly through the use of $^{14}$C-labeled assimilates. If both an inhibitor and a promoter were translocated to the bud where they competed for control over development, it is certainly not apparent why long-day tissue has to be located between the induced leaf and the bud, making hypothesis 4 unlikely. Thus, of the explanations apparent to us, only hypothesis 5, at best a rather nebulous and unsatisfying concept, remains.

Further experiments were carried out to characterize the long-day inhibitory effect. Although the inhibition moves only slightly (tissue must be between induced leaf and bud—experiments 1, 2, 6, 7, and 12), it apparently moves some distance out of the leaf (probably from leaf 2 to the stem). The effect
will not pass through dead tissue, although the transpiration stream will. Inhibition is produced in iron-deficient tissue, although florigen is not.

In every respect studied by us, the kinetics of long-day inhibition proved to be exactly opposite to those of florigen production. The light intensities just capable of inhibiting florigen production are also just capable of promoting inhibition. The critical night for promotion is essentially the same as the critical night limiting inhibition. The kinetics of a light break are essentially the same for inhibition of florigen production and for promotion of inhibition. It seems quite clear that, when the leaf is not producing florigen, it is actively producing some inhibitory effect.

A number of questions and consequences become apparent from these experiments. Some of these suggest further experimentation.

First, how can the close interrelationships between the kinetics of florigen synthesis and long-day inhibition be related to time measurement in the flowering process? Evidence indicates that Xanthium plants oscillate in their sensitivity to light through two phases (21, 23). When plants are in one phase, light is promotive for flowering; when in the other phase, light is inhibitory. Future study might determine the relationship between this observation and those presented in this paper.

Second, it has long been known (18) that grafting experiments designed to show transmission of florigen usually do not succeed unless leaves are removed from the receptor plants. It has been assumed that this is because photosynthesizing leaf tissue prevents the receptor plants from acting as sinks. Threshold light intensities of this response should be studied. Perhaps receptor plants on long-day will not receive the floral stimulus because of the inhibitory effects produced in their leaf tissue.

Third, our results may help to explain the difficulties encountered in development of a bioassay for florigen. If the long-day inhibitory effect is a destruction of florigen, then florigen applied to plants on long-day should never be effective. Hodson and Hammer (17) and Carr (2) have reported that bioassays are more effective in the presence of gibberellin. Does the gibberellin inactivate the long-day inhibitory effect? We have performed preliminary experiments to test this question, and results were negative.

Fourth, our results suggest improved methods for a florigen bioassay. Substances should be applied to plants between long-day leaf tissue and the bud, or all leaves should be removed, or substances might be applied to iron-deficient plants on short-day (neither florigen nor inhibitor should be produced in such plants).

**LITERATURE CITED**


