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# Translocation Patterns in *Xanthium* in Relation to Long Day Inhibition of Flowering[1]

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## ABSTRACT

**The nature of long day inhibition of flowering in the short day plant *Xanthium strumarium* L. was studied by correlating the flowering response with the translocation of $^{14}C$-assimilates from induced leaves or parts thereof to the shoot tips.**

**In contrast to an earlier study by Gibby and Salisbury (Plant Physiol 1971 47: 784-789) no inhibitory effect of an immature leaf in long day on the flower-promoting effect of an induced leaf was detected. When the stimulus moved in the basipetal direction, mature leaves in long day were inhibitory to flowering and at the same time reduced the amount of $^{14}C$-photosynthate that accumulated in the receptor buds.**

**Inhibition of flowering was observed when the apical half of a single leaf was induced and the basal half kept under long day conditions. Induction of a lateral leaf half, with the other half remaining in long day, resulted in a normal flowering response. More $^{14}C$-photosynthate was translocated to the shoot tip from the basal than from the apical leaf half.**

**Removal of the noninduced basal leaf half except for the midrib and major veins, cutting the basal half along the midrib, or keeping the basal half in darkness, resulted in normal flowering. In all three treatments the amounts of $^{14}C$-assimilates that accumulated in the shoot tips also increased, presumably because competing export of nonlabeled assimilates from the basal leaf halves was diminished or eliminated.**

**Autoradiography indicated that $^{14}C$-assimilates produced in the apical half were channeled through the basal half in the major veins and midrib to the petiole without partitioning back into the mesophyll. Since the veins and midrib in the basal half were by themselves not inhibitory to flowering, it is unlikely that the floral stimulus was inactivated in the long day tissue. When the basal half was labeled with $^{14}CO_2$, there was no indication in autoradiograms that $^{14}C$-labeled assimilates moved in the blade in the apical direction.**

**As a whole these results demonstrate that transmission of the floral stimulus and translocation of photosynthate are correlated, so that at least part of the long day inhibition in *Xanthium* can be explained in terms of translocation effects. However, the involvement of a transmissible inhibitor produced in long day tissue cannot be ruled out.**

It is generally assumed that the floral stimulus generated in the leaves of photoperiodic plants moves in the phloem along with the photosynthates. Noninduced leaves that are positioned between the induced ones and the receptor buds as a rule have an inhibitory effect on flowering. The simplest interpretation of this phenomenon is that the noninduced leaves provide an alternate source of photosynthate and thus prevent the floral stimulus from reaching the receptor buds (11). Experimental evidence to support this hypothesis was provided by Chailakhyan and Butenko (2) in autoradiographic studies of $^{14}C$-photosynthate movement and accumulation in *Perilla*, and in a subsequent more quantitative study with the same plant by King and Zeevaart (10). There are, however, other experimental results, grouped under "obscure cases" by Lang (11) that cannot be so readily interpreted in terms of translocation effects. Most notable among these are experiments in which an inductive treatment was given either to the basal or to the apical half of a leaf. Chailakhyan (1) working with the SDP[4] *Perilla*, Harder *et al.* (9) with the SDP *Kalanchoë*, and Gibby and Salisbury (7) with the SDP *Xanthium* found that while induction given to the basal half of a leaf caused flowering, the effect of the induced apical half was strongly inhibited or completely suppressed by the noninduced tissue. The inhibition in *Perilla* was entirely removed by keeping the basal half in continuous darkness (1). Gibby and Salisbury (7) concluded that LD inhibition of flowering in *Xanthium* by the noninduced basal leaf half is a localized condition that intercepts and inactivates the floral stimulus, hence the requirement that in order to be inhibitory, LD tissue must be located between the induced leaf and the receiving bud.

There is also evidence that immature leaves which might be expected to attract photosynthate to a shoot do actually inhibit flower formation. For example, immature leaves left on the receptor branch of a two-branched *Xanthium* plant partially inhibited the flowering response caused by the donor branch (14). Gibby and Salisbury (7) reported that an immature *Xanthium* leaf kept in LD completely suppressed the flower-promoting effect of a leaf in SD. It is possible that in these cases the immature noninduced leaves were still sinks that attracted assimilates flowing to the shoot tip. Thus, the floral stimulus would be diverted from the receptor bud.

The objective of this study was to investigate the possible involvement of translocation effects in LD inhibition of flowering in *Xanthium*. Induced leaves or parts thereof were labeled with $^{14}CO_2$, and the movement of $^{14}C$-labeled assimilates out of the leaves to the shoot tips was determined by autoradiography and by counting the amount of radioactivity that accumulated in the shoot tips. In parallel experiments the flowering response was measured in plants that received the same photoperiodic treatments as the labeled plants. Our results indicate that the flowering response is correlated with the amount of $^{14}C$ activity that was translocated from induced tissue to the receptor shoot tips.

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[4] Abbreviations: LD: long day(s); SD: short day(s); LDP: long day plant(s); SDP: short day plant(s).

## MATERIALS AND METHODS

**Plant Material.** *Xanthium strumarium* L., Chicago strain, was grown in a greenhouse and maintained in the vegetative state by extending natural daylight to a photoperiod of 20 hr as described before (17). The plants were usually 2 months old when used for experimentation. Following Salisbury's terminology (7, 18), the smallest leaf longer than 1 cm (midrib) was called leaf 1, the next largest, leaf 2, etc. Plants were selected with leaf 3 between 7 and 8 cm, the stage at which *Xanthium* leaves are maximally sensitive to photoperiodic induction (18). At that time the laminae of leaves 2 and 3 had a mean upper surface area of 14 $cm^2$ and 44 $cm^2$, respectively. Leaf area was determined by making a print of a blade with a photocopying machine and measuring the area with a planimeter. On the same day, but prior to the beginning of the inductive period, the plants were trimmed to leaf 3, or as required for the experimental design. They were then moved to a growth chamber until the time of induction.

**Photoperiodic Treatments.** The growth chamber was maintained at a temperature of 23 C and a relative humidity of around 60%. A photoperiod of 20 hr was maintained, except during inductive treatments, with 12 hr of high intensity light from fluorescent tubes (FR96T12/CW/VHO/135) and 40 w incandescent lamps (total irradiance 12.5 mw $cm^{-2}$), followed by an 8-hr period of low intensity light from the incandescent lamps only (irradiance 3 mw $cm^{-2}$).

Leaf 3 or part of it was given a 16-hr dark period by covering it with an envelope of black paper fastened with two large paper clips. During the entire inductive treatment of leaf 3 (4 PM to 8 AM) the incandescent lamps were left on, and the fluorescent tubes were left off. Following induction the plants remained in the growth chamber under the 20-hr photoperiods. The terminal buds were dissected 9 days after the end of induction, and flowering was scored according to Salisbury's system of floral stages (18). Ten plants were included in each treatment. Each experiment was repeated at least twice with similar results.

**Labeling and Translocation Experiments.** Unless otherwise stated, leaves or parts thereof were labeled with $^{14}CO_2$ at the end of the 16-hr inductive dark period. $^{14}CO_2$ was generated from 2 mg of $Ba^{14}CO_3$ (59.7 mCi/mmol) and 3 mg of nonradioactive $BaCO_3$ in a three-necked flask by injection of 20% lactic acid through a serum vial cap, and circulated for 8 min over six leaves placed together in a Plexiglas chamber (17.5 × 31 cm). Light from two 250 w flood lamps was filtered through a 5-cm layer of water to give an irradiance of 16 mw $cm^{-2}$ below 690 nm at the leaf surface. During the labeling procedure parts of the laminae were kept in darkness to prevent them from being labeled. The black envelopes used for this were covered with aluminum foil to reduce heat absorption by the black paper. After labeling, the foil-covered envelopes (if applied) were removed and the plants were returned to the growth chamber with both the fluorescent and incandescent lights turned on. During and after labeling the shoot tips and stems were covered with aluminum foil to prevent assimilation of $^{14}CO_2$ released during respiration. Plants were harvested 4 to 6 hr after the start of $^{14}CO_2$ uptake. In most experiments the terminal shoot tip (3–6 mg dry wt) and 1-cm stem segments (dry wt 25–90 mg) 10 and 20 cm below leaf 3 were harvested. In the experiment of Table II the axillary bud at node 7 and 1-cm segments above and below that node were excised. The samples were immediately frozen in liquid $N_2$, lyophilized, and their dry wt determined. They were combusted in Packard combustocones with Whatman CC 41 ashless cellulose powder (total wt of combusted sample, 300–500 mg) in a Packard model 306 Tri-Carb sample oxidizer for 0.5 min, and then counted in vials containing a mixture of 9 ml of Packard Carbo-Sorb II and 12 ml of Permafluor V. By spiking several samples before and after burning with $^{14}C$-hexadecane, the sample oxidizer was found to be 97% efficient in recovering the $^{14}CO_2$ generated. The samples were counted in a Packard model 3375 Tri-Carb liquid scintillation spectrometer for 10 min. The counting efficiency was determined, and all data were converted to dpm/mg dry wt.

Since only six leaves could be accommodated in the assimilation chamber at a time, as a rule two treatments of three leaves were compared in a labeling experiment. Because of this small number, each experiment was repeated several times. Although the absolute amounts of radioactivity detected varied from one experiment to another, the same trends were observed in all experiments.

The relative amounts of $^{14}CO_2$ fixed in light and darkness were determined by exposing leaves to $^{14}CO_2$ of low specific radioactivity generated from $Ba^{14}CO_3$ (0.05 mCi/mmol) as described above. Immediately after labeling, four discs with a diameter of 1.2 cm were punched from the middle part of a leaf, two from each side of the midrib in a criss-cross pattern, so that duplicate samples with two discs each were obtained from each leaf. The samples were combusted and counted as above.

**Autoradiography.** Leaves were labeled with $^{14}CO_2$ of high specific radioactivity as described above and allowed to transport $^{14}C$-assimilates until harvested. Labeled leaves were excised from the plants and immediately dropped into liquid $N_2$ while flattened between two pieces of cardboard that were secured with rubber bands. The frozen leaves were lyophilized overnight. They were then kept in a humid atmosphere for about 15 min to reduce brittleness, mounted, and exposed to Kodak Industrial M-54 x-ray film for 2 to 6 days.

In addition to the experiments on $^{14}C$-assimilate transport, the movement of applied $^{14}C$-sucrose out of a leaf was followed, because sucrose is the major transport sugar in *Xanthium* (unpublished results). In one experiment 5 $\mu$Ci of $^{14}C$-sucrose (4.3 mCi/mmol) in water with Tween 20 and 5% ethanol were applied to the apical half of leaf 3 within an area of 1 $cm^2$ bounded by lanolin. The area was sealed with a cover glass to avoid evaporation. Transport was allowed to continue for 24 hr. In this case autoradiograms were prepared not only of the leaf, but also included the stem with the shoot tip above leaf 3.

## RESULTS

### INDUCTION OF A SINGLE LEAF WITH OTHER LEAVES REMAINING IN LD

**Upward Movement of Floral Stimulus.** Induction of leaf 3 in the presence of one or more leaves in LD did not significantly inhibit the flowering response of the terminal shoot apex, regardless of whether the LD leaves were between the induced leaf and the shoot apex, or below the former (Table I). This was the case also when leaf 3 was exposed to one 16-hr dark period and leaf 2, positioned between the induced leaf and the shoot apex, was kept in continuous light. On this point our results are at variance with those of Gibby and Salisbury (7, Fig. 2) who reported that leaf 2 kept in LD completely suppressed the flower-inducing effect of leaf 3. Since we failed consistently in detecting any flower-inhibiting effect of noninduced leaves positioned either above or below donor leaf 3, only preliminary translocation studies with $^{14}C$-labeled photosynthate were carried out. When leaf 3 was labeled, the presence of leaf 2 on LD did not reduce accumulation of $^{14}C$ activity in the shoot tip. However, a large amount of radioactivity did accumulate in leaf 2, indicating that this young leaf was still a sink for photosynthate. In plants that retained leaves 3 through 7, labeling of leaf 4 with $^{14}CO_2$ resulted in at least as much $^{14}C$-labeled photosynthate moving into the shoot tips as when

Table I. *Effect of LD Leaves on the Flowering Response of the Terminal Bud Induced by a Single Leaf in SD.*

No. 3 leaves were exposed to one 16-hr dark period
No. 2 leaf blades between 4 and 5.5 cm
No. 3 leaf blades between 7 and 8 cm
No. 4 leaf blades between 9 and 10 cm

| Leaves kept on plants | Floral stage |
|---|---|
| No. 3 | 5.2 ± 0.36[a] |
| No. 2 and 3 | 5.2 ± 0.25 |
| No. 3 and 4 | 5.0 ± 0.00 |
| No. 1 through 6 | 4.9 ± 0.31 |

[a]Standard deviation of the mean

induced leaf 3 was labeled, even though leaf 4 had no inhibitory effect on flowering (Table I).

**Downward Movement of Floral Stimulus.** In this experimental set-up the terminal apex was removed and an axillary shoot at node 7 (counting downward) functioned as the receptor bud. Leaf 3 was given a 16-hr dark period, and one or two LD leaves were kept at nodes 5 and 6. It can be seen (Table II) that in this case mature LD leaves located between the induced leaf and the receptor bud had a marked inhibitory effect on flowering. In order to determine if the LD leaves interfered with the translocation of the floral stimulus, $^{14}CO_2$ was fed to the induced leaf, and the bud, as well as 1-cm stem sections of the internodes above and below node 7, were harvested and analyzed for radioactivity. It is clear from the results in Table III that the presence of LD leaves did indeed strongly reduce the amount of $^{14}C$-assimilates that accumulated in the bud. Accumulation of radioactivity in the stem was also reduced, but much less so than in the bud, by the presence of LD leaves.

### INDUCTION OF VARIOUS PARTS OF A SINGLE LEAF

**Flowering Response.** Results of an experiment in which various parts of the lamina were given SD treatment (Fig. 1), confirm those obtained by other workers (1, 7, 9). Induction of the basal leaf half only was as effective as inducing the whole blade, while induction of the apical half, with the basal half in LD, resulted in a much reduced flowering response. Flower induction of a lateral leaf half was not significantly reduced by exposure of the other half to LD (Fig. 1). Thus, a noninduced leaf half exerted a strong flower-inhibiting effect only when positioned between the induced part and the shoot apex. Further experiments were designed to find treatments that could reduce or eliminate this LD inhibition of flowering. It had been shown in earlier work (7, 9) that removal of the basal, noninduced leaf half restored flowering. Our results (Table IV) confirm this and further show that this treatment became less effective, the later the LD base was cut away.

In the next experiment the apical half was given a 16-hr inductive dark period, after which the basal half was darkened for 24 hr, with a 5-min light interruption after 8 hr to prevent this tissue from itself inducing flower formation. The results (Table V) show that darkening of the LD basal leaf half effectively restored flowering. Since the controls were vegetative, it follows that flowering was in response to stimulus generated in the apical half and translocated to the shoot tip.

Separating the induced apical leaf half from the LD basal tissue by cutting the blade at right angles to the midrib did not promote flowering. In fact, in all experiments of this type the flowering response was even further reduced. However, severing the basal leaf half from the midrib resulted in a normal flowering response (Fig. 2). Conversely, it was expected that severing the

Table II. *Flower-Inhibiting Effect of LD Leaves Positioned between the Induced Leaf and the Receptor Bud.*

In order to release the axillary buds from apical dominance, the plants were decapitated above leaf no. 3 two days prior to induction, and leaf no. 4, as well as all axillary buds, except at node no. 7, were removed. Further defoliation took place immediately prior to the inductive treatment of leaf no. 3

| Leaves in LD | Floral stage |
|---|---|
| None | 5.5 ± 0.27 |
| No. 5 | 2.7 ± 0.60 |
| No. 5 and 6 | 0.8 ± 0.39 |

Table III. *Inhibitory Effect of LD Leaves on Accumulation of $^{14}C$-Assimilates in Axillary Buds and Stem Tissue after Labeling of the SD Leaf.*

Plants were prepared as in Table II. Axillary buds at node 7 harvested 6 hr after labeling of leaf 3.
Two plants per treatment.

| Leaves in LD | Radioactivity in: Axillary shoot at node 7 | Stem above node 7 | Stem below node 7 |
|---|---|---|---|
| | dpm/mg dry wt. | | |
| None | 160,950 | 31,950 | 17,120 |
| No. 5 | 26,810 | 21,270 | 13,060 |
| No. 5 and 6 | 23,640 | 15,720 | 9,740 |

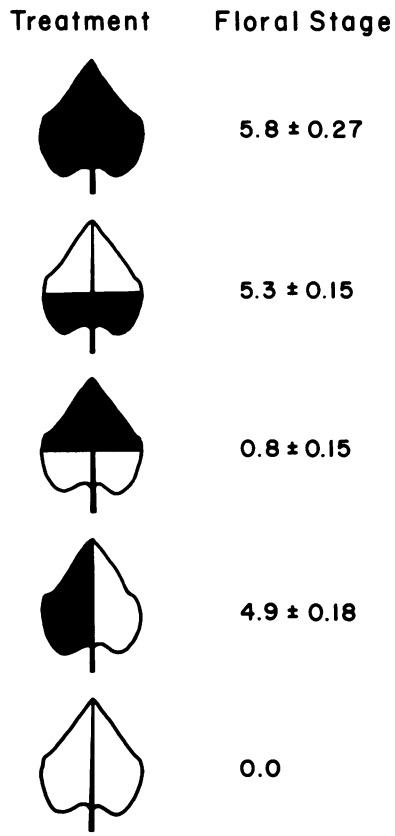


FIG. 1. Flowering response in *Xanthium* after exposing different parts of leaf to 16 hr of darkness. In this and following figures, black areas of laminae were exposed to a 16-hr dark period; white areas were kept in LD.

Table IV. *Effect of Removing the LD Basal Leaf Half at Different Times on the Flowering Response Induced by the Apical Half.*

0 hr indicates beginning of 16-hr dark period to which the apical leaf half was exposed.
Major veins and midrib of basal half were retained.

| LD basal leaf half removed at .. hr | Floral stage |
|---|---|
| 0 | 4.5 ± 0.17 |
| 16 | 4.2 ± 0.33 |
| 24 | 3.6 ± 0.31 |
| 40 | 2.9 ± 0.41 |
| ∞ | 1.2 ± 0.33 |

Table V. *Effect of Darkening the LD Basal Leaf Half on the Flowering Response Induced by the Apical Leaf Half.*

| Apical leaf half | Basal leaf half | Floral stage |
|---|---|---|
| 16 hr darkness[a] | Light | 0.7 ± 0.21 |
| 16 hr darkness[a] | 24 hr darkness[b] | 4.7 ± 0.26 |
| Light | Light | 0.0 |
| Light | 24 hr darkness[b] | 0.0 |

[a] Apical leaf halves in darkness from 0 to 16 hr.

[b] Basal leaf halves darkened from 16 to 40 hr with a 5-minute light interruption at 24 hr.

basal *induced* leaf half from the midrib would diminish or eliminate flowering. As shown in Figure 3, this was indeed the case but the cuts along the midrib had to be extended at least 1 cm into the LD tissue to suppress flowering completely.

**Autoradiography.** When $^{14}CO_2$ was fed to a leaf, the half exposed to light during labeling became highly radioactive (Fig. 4). However, the part kept in total darkness during labeling also showed a faint image, even when frozen as soon as 15 min after the beginning of labeling (Fig. 4A). Since leaves that were not exposed to $^{14}CO_2$ gave no image on x-ray film, the low level of radioactivity in leaf halves kept in darkness must have been the result of $^{14}CO_2$ dark fixation. Combustion of leaf discs taken from leaves in light or darkness, and subsequent combustion and counting of the radioactivity indicated that fixation of $^{14}CO_2$ in the dark amounted to about 0.5% of that in the light.

In a time course study of movement of $^{14}C$-assimilates out of the apical leaf half, it was evident that 15 min after the beginning of $^{14}CO_2$ feeding, the basal half gave a rather uniform, but very weak image on the x-ray film (Fig. 4A), as the result of dark fixation of $^{14}CO_2$ (see above). Radioactivity began to appear in the midrib and major veins after 30 min and reached a maximum after 2 to 4 hr (Fig. 4B). The labeling in the veins and midrib of the basal leaf half and in the petiole had clearly declined after 8 hr. During this transport period the radioactivity remained in the major veins, the midrib, and the petiole, and did not appear in the mesophyll (Figs. 4B and 5A). However, when the basal leaf half was darkened, radioactivity also appeared in the smaller veins in the mesophyll (Fig. 5B).

Separating the apical leaf half from the basal half by cutting the mesophyll at right angles to the midrib eliminated the major veins as channels for movement of the assimilates. As shown in Figure 5C, radioactivity in the basal half was in this case restricted to the midrib. Some autoradiograms also showed a slight movement of radioactivity out of the midrib into the veins of the basal half.

When the basal leaf half was severed from the midrib (Fig. 5D), $^{14}C$-labeled assimilates could only be translocated out of the apical half via the midrib. At the same time $^{14}C$-photosynthate was channeled from the apical to the basal leaf half and accumulated in the triangular network of veins circumscribed by the two major veins that fuse with the midrib at the base of the blade (Fig. 5D). As compared to a darkened leaf base very little radioactivity accumulated in the mesophyll (compare Fig. 5, B and D), and the proximal lobes of the blade remained free of $^{14}C$ activity.

There was never any indication in autoradiograms that $^{14}C$-assimilates moved from the basal leaf half in the apical direction (Fig. 4C). This was the case even when the basal half was separated from the midrib prior to labeling (Fig. 4D). Nevertheless, as shown below some radioactivity did move from such basal leaf halves to the shoot tip (see Table VIII), but the amount of radioactivity involved was apparently insufficient for detection in autoradiograms.

$^{14}C$-Sucrose applied to the apical leaf half moved out of the lamina via the nearest veins and midrib, and accumulated to a

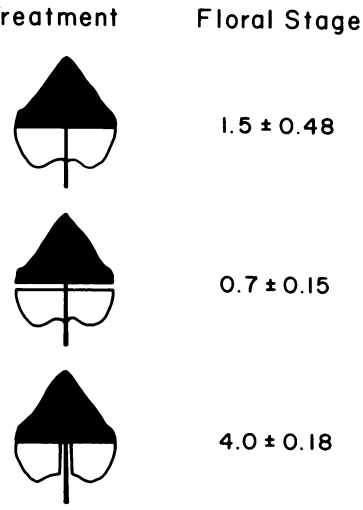


FIG. 2. Effect on flowering response of separating LD basal leaf half from SD apical half, or from midrib. Cuts were made at end of 16-hr dark period administered to apical half.

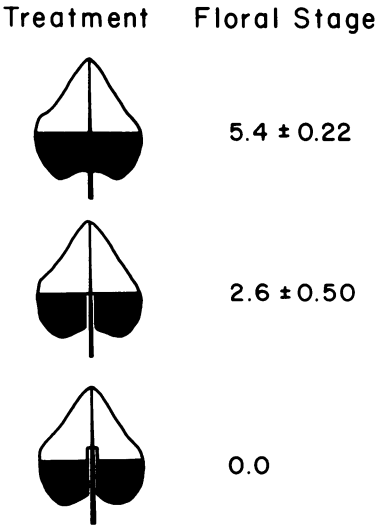


FIG. 3. Effect on flowering response of separating SD basal leaf half from midrib. Cuts were made at end of 16-hr dark period given to basal leaf half. In third treatment, the cut along midrib was extended 1 cm into LD apical leaf half.

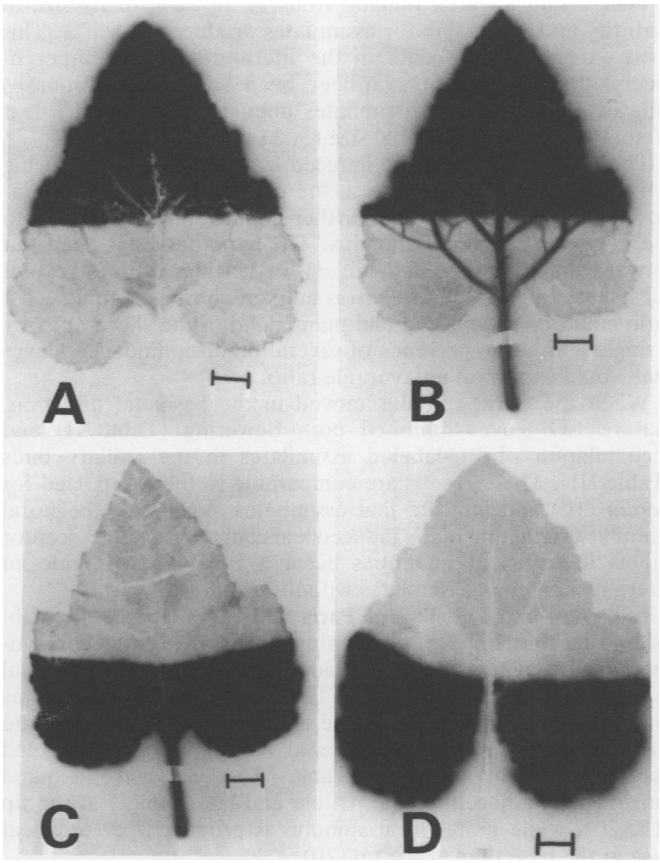


FIG. 4. Autoradiograms of *Xanthium* leaves following $^{14}CO_2$ fixation. During labeling either apical or basal leaf half was exposed to light. A: SD apical half in light; frozen after 15 min. B: As A, but frozen after 2 hr. C: SD basal half in light; frozen after 5 hr. D: SD basal half was separated from midrib and the latter was wrapped in aluminum foil prior to labeling. Frozen after 6 hr.

considerable extent in the shoot tip. Contrary to Gibby's observations (6, p. 37) with $^{14}$C-glucose applied to the apical leaf half, $^{14}$C-sucrose did not accumulate in the basal part of the lamina. $^{14}$C-Sucrose applied to the tip of a bean leaf also moved out of the blade via the midrib; only if the midrib had been severed, did label move into the veins (16).

**Accumulation of $^{14}$C-Assimilates in Shoot Tips.** In these studies either the basal or apical leaf half was kept in light during $^{14}CO_2$ feeding, and the amount of radioactivity that accumulated in the shoot tip and stem was determined. Various treatments known to inhibit or promote flowering (see above) were applied to the leaves in order to see if translocation from the induced leaf half was correlated with the flowering response.

First, export from the apical and basal leaf half was compared. The data in Table VI show that more radioactivity from the basal than from the apical half accumulated in the shoot tips. However, the amounts of radioactivity exported by the respective halves to the shoot tips were not proportional to the flowering response (Fig. 1) observed.

Second, translocation from the induced apical leaf half was compared directly after induction when the LD basal half was removed, darkened, or separated from the apical half, or from the midrib. The results in Table VII indicate that the amounts of $^{14}$C-assimilates exported to the shoot tip were correlated with the flowering response in all treatments. For example, removal or darkening of the LD basal leaf half enhanced export of $^{14}$C-photosynthate (Table VII) and also promoted flowering (Tables IV and V). Cutting the mesophyll between the apical and basal half reduced the flowering response (Fig. 2), and also lowered the amount of radioactivity that accumulated in the shoot tip (Table VII). On the other hand, cutting the LD basal half along the midrib had a strong flower-promoting effect (Fig. 2), and increased the amount of radioactivity in the shoot tip 4.5 times.

Third, the movement of assimilates from the SD basal leaf half was studied when the leaf was cut along the midrib. As shown in Table VIII, a small amount of radioactivity was translocated to the shoot tip, even when the cuts were extended 1 cm into the LD half. At least qualitatively, there was a correlation between the flowering response (Fig. 3) and the radioactivity in the shoot tips (Table VIII).

## DISCUSSION

**Effects of Leaves in LD.** No evidence was obtained in the present investigation that the immature leaf 2 maintained in LD

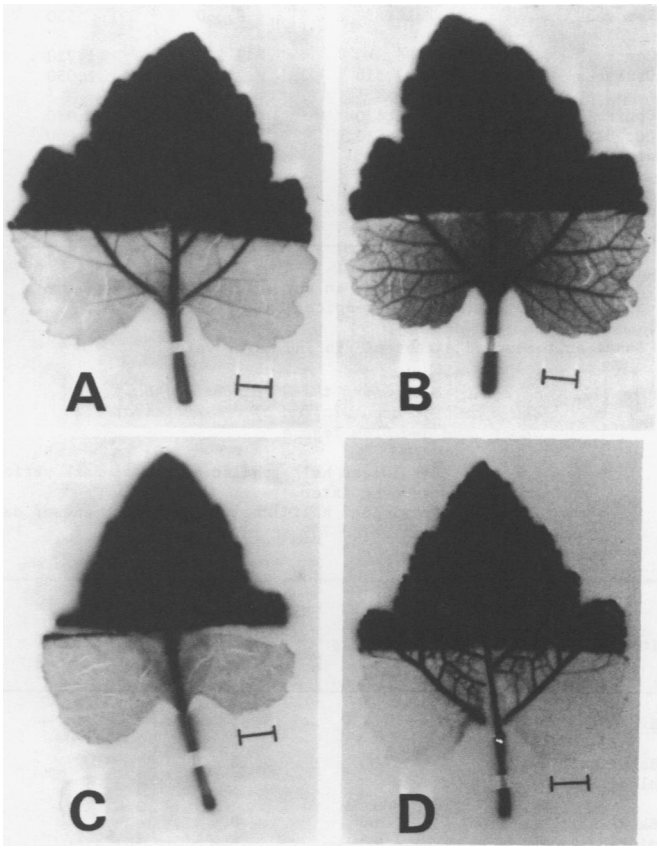


FIG. 5. Autoradiograms of *Xanthium* leaves following $^{14}CO_2$ fixation with SD apical halves in light. A and B: Following labeling, basal leaf half was kept in light (A) or darkness (B). Frozen after 6 hr. C: Blade cut at right angles to midrib after labeling. Frozen after 5 hr. D: Basal leaf half cut along midrib after labeling. Frozen after 3 hr. Horizontal bar with each leaf indicates 1 cm.

Table VI. *Accumulation of $^{14}$C-Assimilates in Shoot Tips and Stem Tissue after Labeling the Apical or Basal Leaf Half.*

Harvested 4 hr after labeling. Three plants per treatment.

| Induced and labeled | Shoot tip | Radioactivity in Stem at 10 cm | Stem at 20 cm |
|---|---|---|---|
| | | dpm/mg dry wt. | |
| Apical half | 52,420 | 3,220 | 1,600 |
| Basal half | 103,250 | 6,130 | 5,440 |

Table VII. *Influence of Various Treatments Given to the LD Basal Leaf Half on Accumulation of $^{14}C$-Assimilates in Shoot Tips and Stem Tissue after Labeling of the Apical Leaf Half.*

Apical leaf halves given 16-hr dark period. Treatments started at end of darkness. Harvested 4 hr after labeling. Three plants per treatment. Since the experiments were performed at different times, data can be compared only between paired treatments.

| Treatment of basal leaf half | Radioactivity in Shoot tip | Stem at 10 cm | Stem at 20 cm |
|---|---|---|---|
| | | dpm/mg dry wt. | |
| Control[a] | 147,740 | 4,860 | 3,760 |
| Removed[b] | 216,830 | 2,360 | 550 |
| Control[a] | 86,080 | 3,290 | 1,720 |
| Darkness | 157,510 | 970 | 1,050 |
| Control[a] | 36,470 | 4,940 | 2,970 |
| Cut between apical and basal half | 24,610 | 2,960 | 800 |
| Control[a] | 50,570 | 2,740 | 1,390 |
| Cut along midrib | 227,740 | 3,070 | 1,130 |

[a]Basal half not cut or removed in any way, but exposed to the same light conditions as the apical half after labeling.

[b]Basal half removed except midrib and major veins.

Table VIII. *Effect of Separating the SD Basal Leaf Half from the Midrib on Accumulation of $^{14}C$-Assimilates in Shoot Tips and Stem Tissue.*

Expt. 1: Basal leaf half labeled at end of dark period and harvested 6 hr later.
Expt. 2: Basal leaf half labeled 4 hr after end of dark period and harvested 7.5 hr later.

| Treatment | Radioactivity in Shoot tip | Stem at 10 cm | Stem at 20 cm |
|---|---|---|---|
| Expt. 1 | | | |
| Basal half cut along midrib | 630 | 10 | 0 |
| Basal half cut along midrib + 1 cm | 100 | 0 | 0 |
| Expt. 2 | | | |
| Basal half cut along midrib | 16,790 | 530 | 100 |
| Basal half cut along midrib + 1 cm | 4,090 | 130 | 30 |

inhibited flower initiation when leaf 3 was given an inductive treatment (Table I). This was also the case when leaf 2 was exposed to light of high intensity during induction of leaf 3 (unpublished results). Gibby and Salisbury (7) reported that leaf 2 completely inhibited the action of the induced leaf 3, while Gibby (6, Fig. 3) found only 50% inhibition. There is no explanation for these contradictory results at present, since the same strain of *Xanthium* was used, and leaves 2 and 3 were in the same developmental stage in both investigations. We have been in touch with Salisbury and Gibby and are jointly seeking an explanation for the opposite effect of the no. 2 *Xanthium* leaf in the two laboratories.

Leaf 2 had an area ranging from 11 to 22% of its size at maturity and was a sink for assimilates produced in leaf 3. This is as expected, since data in the literature for a number of dicotyledonous species (sugar beet, bean, soybean, and squash) indicate that import of assimilates does not cease until a leaf attains 45 to 50% of its final size (5, 21–24).

The observation that one induced leaf is capable of inducing a normal flowering response in the presence of several noninduced leaves (Table I) has a further implication with respect to the nature of the floral stimulus. The hypothesis has been put forward that flowering depends on a particular ratio of several substances (*e.g.* 25, p. 248). It is unlikely, however, that such a ratio could be established and maintained in the shoot apex by a single leaf in the presence of several other noninduced leaves that would export an unfavorable ratio.

When the floral stimulus moved in the basipetal direction, mature LD leaves inhibited both flowering (Table II) and accumulation of $^{14}C$-labeled assimilates in the axillary buds (Table III). These results are comparable to those reported for *Perilla* (10) and indicate that assimilates along with the floral stimulus originating in the induced leaf cannot reach the receptor shoots in sufficient quantities because LD leaves provide an alternate supply of assimilates without stimulus.

**Induction and Labeling of Parts of Leaves.** It is well established that the ability of developing leaves to export photosynthate begins at the tip of the blade and progresses in basipetal direction as the leaf expands (5, 13, 23). At the time leaf 3 was induced in the present study, the area was 50% of its ultimate size, and both the apical and basal half exported $^{14}C$-labeled assimilates to the shoot tip (Table VI). Such leaves expand most rapidly adjacent to the petiole and least rapidly at the tip (15, 20), while more floral stimulus is produced per unit leaf area at the base than at the tip (20).

In the light $^{14}C$-assimilates were channeled out of the apical leaf half through the major veins and midrib without migrating to other portions of the blade (Figs. 4B and 5A). This makes it unlikely that LD inhibition is based upon inactivation of the stimulus in the basal half, unless one further postulates (7) that the inhibitor moved into the veins and destroyed the floral stimulus in this tissue. Since removal of the mesophyll (Table IV) or cutting the basal half along the midrib (Fig. 2) results in normal flowering, the midrib and veins are not inhibitory by themselves. Thus, the inhibitory LD effect would have to have originated in the mesophyll. However, it is hard to visualize how an inhibitor would move from LD mesophyll to the midrib and veins and then remain localized in these conducting tissues. Darkening the basal half caused partitioning of $^{14}C$-assimilates back into the basal tissue (Fig. 5B) which, if an inhibitor were involved, should enhance the possibility of stimulus inactivation. Yet, the flowering response was normal (Table V), indicating that the floral stimulus moved through the noninduced tissue without being impeded. In view of these observations we consider it unlikely that LD inhibition in *Xanthium* is a localized condition.

Any treatment that decreased or eliminated the flower-inhibiting effect of the LD basal half at the same time increased the amount of $^{14}C$-assimilates that was translocated from the induced apical half to the shoot tip (Table VII). However, as already noticed for whole plants (see above), there was no strict quantitative relationship between the flowering response and the amount of radioactivity that accumulated in the shoot tip. This was also true when the basal half was induced and cut along the midrib (Table VIII). The question can, of course, be raised to what extent the distribution of $^{14}C$-radioactivity following a pulse with $^{14}CO_2$ after the inductive period mirrors long term partitioning of photosynthate. This remains an important unanswered question because export of floral stimulus from an induced *Xanthium* leaf continues for many hours (18, 19),

while our translocation experiments lasted only 4 to 6 hr.

The hypothesis that translocation effects can account for LD inhibition in *Xanthium* was rejected (7) on the basis that very low light intensities (below the photosynthetic compensation point) were sufficient for LD inhibition. However, the low light intensity to the basal leaf half was given concurrently with the 16-hr dark period to the apical half (7), while the entire leaf was under light of high intensity following induction; *i.e.* during that period when the floral stimulus is exported from the leaf (18, 19) and when translocation effects would be expected. In our experiments translocation obviously played an important role in determining the flowering response. For example, cutting the LD basal leaf half along the midrib allowed translocation from the induced apical half while blocking it from the noninduced half (Figs. 2 and 5D). When SD and LD halves were separated, translocation from the apical half again could only take place via the midrib, but since export from the basal half was not interrupted, inhibition of flowering was observed (Figs. 2 and 5C). The question then is: does LD inhibit flowering because the LD leaf area is supplying assimilates to the shoot apex, resulting in a dilution of the floral stimulus, or is it caused by a mobile inhibitor that counteracts the effect of the floral stimulus in the shoot apex? The general trend of our quantitative data obtained in short term labeling and translocation studies (see above) supports the former possibility, but the involvement of a transmissible inhibitor in flowering of *Xanthium* cannot be ruled out and can certainly account for our results. Evidence in the literature for a transmissible inhibitor is much less abundant than that for a floral stimulus, but it has been reported for the SDP *Fragaria* (8) and for the LDP *Lolium temulentum* (3, 4). In grafting experiments the transmission of a flower inhibitor from the LDP *Nicotiana silvestris* under SD to a dayneutral variety of tobacco has recently been demonstrated (12).

Pertinent to the question of a transmissible inhibitor *versus* translocation and dilution effects is Searle's experiment (20) in which a donor leaf of *Xanthium* was split along its midrib and petiole, and one side was induced with the other remaining under LD. When the SD and LD leaf areas were equal, flowering was normal; however, when the induced half was trimmed, so that the LD area was four times larger than the SD area, flowering was inhibited. This inhibition was attributed to a LD inhibitor (20), but a dilution effect as proposed above also seems possible.

When tissues alike in area and orientation were used for induction and inhibition as in Searle's work (20), and in our experiment with one lateral leaf half in SD (Fig. 1), the effect of the induced part clearly dominated. Why then did the effect of a LD basal half dominate over that of a SD apical half (Fig. 1)? In addition to the possibilities already discussed, part of the answer may lie in the observation that floral stimulus production in the almost mature apical half is less per unit leaf area than in the rapidly expanding basal half (20). Work to assess the contribution of these various processes to LD inhibition of flowering in *Xanthium* is continuing.

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