INFLORESCENCE INITIATION IN *LOLIUM TEMULENTUM* L.

IV. TRANSLOCATION OF THE FLORAL STIMULUS IN RELATION TO THAT OF ASSIMILATES

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Summary

Translocation of labelled assimilates to the shoot apex and other parts of the plant was followed from an upper leaf held in long-day conditions, when lower leaves in short days were either present or removed. Similarly a comparison was made of the distribution of assimilates from an upper long-day leaf and a lower leaf held in short days. The presence of lower leaves did not reduce the movement of assimilates from the upper leaf to the shoot apex, and the lower leaf supplied only a small proportion of the assimilates reaching the shoot apex, although it supplied much to the roots. It is concluded that the previously established inhibitory effect of lower leaves in short days on inflorescence initiation in *L. temulentum* is unlikely to be due to their interference with translocation of the long-day stimulus to the shoot apex, or to their diluting it with assimilates, but rather to their production of a transmissible inhibitor of initiation.

In a further experiment in which plants with only the sixth leaf blade were exposed to photoperiods of various lengths, and in which this leaf blade was removed at various times, it was shown that the critical photoperiod length was about 16 hr, and that within 4 hr of this period sufficient long-day stimulus moved from the leaf blade to cause initiation. From this and other data it is estimated that the minimum rate of movement of the floral stimulus is about 2 cm/hr.

I. Introduction

In an earlier paper (Evans 1960*b*) it was concluded that leaves of *Lolium temulentum* L. exposed to one long day produce a transmissible stimulus to inflorescence initiation, and leaves in short days a transmissible inhibitor of it. This inhibitor presumably interacts with the long-day stimulus at the shoot apex, the site of inflorescence differentiation. A similar dual system of day-length control of initiation may operate in *Pisum sativum* (Barber 1959; Haupt 1961) but, as Haupt points out, grafting experiments do not give clear evidence of the transmissibility of the inhibitory effect. Among long-day plants, apart from *L. temulentum*, there is no established transmissible inhibition by short-day leaves, and Zeevaart (1962, 1963) has questioned the above interpretation of the earlier experiments with *L. temulentum*. He suggests that the inhibitory effect of short-day leaves in these experiments could have been due to their interference with the translocation of the long-day stimulus, or to dilution of the stimulus at the growing point by assimilates translocated from these leaves. Zeevaart suggests that translocation experiments with labelled assimilates, like those carried out by Chailahjan and Butenko (1957) with *Perilla*, should clarify the role of leaves in short-day conditions. We report such experiments here.

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Leaves inserted between the photoperiodically induced leaves and the receptor bud have frequently been shown to have an inhibitory effect on flower initiation, which Lang (1952) suggested was due to their generating a solute stream opposed to that from the induced leaves. He concluded that leaves not inserted between the induced leaves and the receptor bud do not act in this way. For this reason, only leaves below that exposed to the long day were examined for a transmissible inhibitory effect in most of the earlier experiments with *L. temulentum*. In the experiments reported here the effect of lower leaves in short days on translocation to the shoot apex from an upper leaf in long days is examined. Translocation from one of the lower short-day leaves to the apex is also measured, to assess the likely extent of dilution of the floral stimulus by assimilates from these leaves.

It should be noted that these experiments examine the movement of labelled assimilates, and not necessarily that of either the long-day stimulus or the short-day inhibitor. Because the identity of these remains unknown, their movement cannot be followed directly, but it is widely assumed that the floral stimulus moves in the phloem with the carbohydrate stream (Lang 1952). As earlier estimates of the rate of translocation of the floral stimulus yield rates which are considerably slower than those for assimilates, a further estimate is attempted here.

II. Experimental Methods

(*a*) *Environmental Conditions*

All plants were grown in 8-hr days, at 25°C/20°C for the 8-hr light and 16-hr dark periods respectively, until the sixth leaf was fully expanded, when the long-day exposures were made.

At 2 p.m. on the long day, either all tillers or all tillers and all leaf blades except the sixth were removed from the experimental plants.

The standard long day consisted of 8 hr at 25°C under light of 3500 f.c. intensity from fluorescent and incandescent lamps, from 8.30 a.m. to 4.30 p.m., followed by 16 hr at 20°C under illumination of 40 f.c. intensity from incandescent lamps.

In the experiment examining the critical photoperiod length for a single long-day exposure, and the time of translocation of the long-day stimulus, groups of 10–12 plants with only the sixth leaf blade remaining were either removed to a dark room at 19°C, or had the blade of their sixth leaf cut off at the base, at two-hourly intervals during the low intensity light period.

Where the sixth leaf blade was exposed to the long day while lower leaves were held in short days, the plants were placed inside the boxes described previously (Evans 1962) at 4.30 p.m. on the long day, and removed at 8.30 a.m. the following morning.

(*b*) *Labelling Experiments*

Two major labelling experiments were carried out. The first examined the extent to which the presence of lower leaves (1–5) on the primary stem, in short days, affected movement of ^{14}C-labelled assimilates to the shoot apex from the sixth leaf, exposed to one long day. The second examined the relative amounts of ^{14}C-labelled

assimilates supplied to the apex by the fourth and sixth leaves on the primary stem. The sequence of operations for these labelling experiments is shown schematically in Figure 1.

At 9.30 a.m. on the morning after the long-day exposure, the terminal 19 cm length of the leaf blades to be labelled (leaf 6 in the first experiment, leaf 4 or leaf 6 in the second) were enclosed in darkened boxes. $^{14}CO_2$ was generated from $Ba^{14}CO_3$ by the addition of lactic acid, to give an initial CO_2 concentration of 0·2% by volume, containing 500 μc of ^{14}C in each box. After 5 min to allow for uniform diffusion of the $^{14}CO_2$ throughout each box, the covers were removed, exposing the enclosed leaves to light of 3500 f.c. intensity for 30 min. It was subsequently estimated that CO_2 concentrations did not fall below 0·04% by volume in this time, so that photosynthesis was unlikely to have been limited by CO_2. The boxes were then opened,

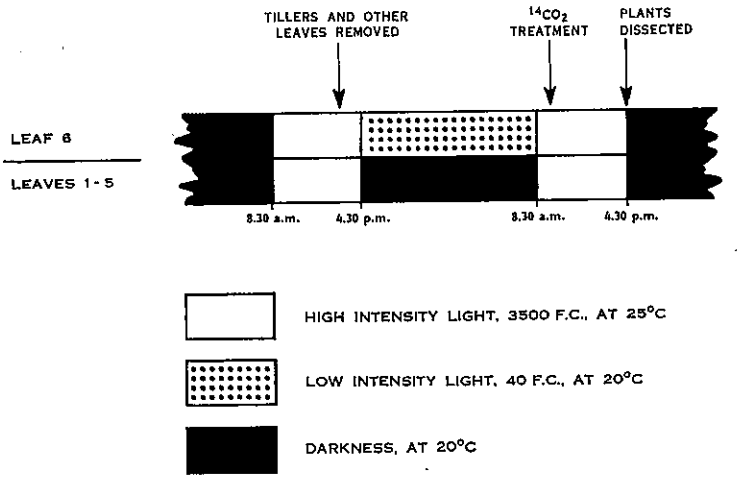

Fig. 1.—Schematic representation of treatments given in the ^{14}C-labelling experiments.

and the plants held under light of 3500 f.c. intensity until they were dissected at 4.30 p.m. Each plant was divided into (i) the leaf blade exposed to $^{14}CO_2$, (ii) older leaf blades, (iii) roots, (iv) the shoot apex, and (v) the remainder of the shoot.

The shoot apices were excised above the level of insertion of the youngest leaf primordium showing any upward growth. Each was about 0·5 mm long and 0·2 mm in diameter. The dissecting knife was rinsed in ethanol and wiped immediately before the final excision of each apex. Because of their small size, apices, in pairs, were plated directly onto aluminium planchets and dried at 70°C. They were then ground in ethanol with a glass pestle and counted directly with a gas flow end-window Geiger–Müller tube. The counts thus obtained were considered to be total counts at infinite thinness for the material.

After drying at 70°C, the other plant parts were weighed and then ground to pass a 40-mesh sieve. The distribution of ^{14}C activity was then determined on paired powdered samples by the method described by O'Brien and Wardlaw (1961). As these latter counts were made at infinite thickness, they are not directly comparable

with the apex counts. When placed on a comparable basis, the apices, although having a very high specific activity (apex 280,000 counts/min; treated leaf 3200 counts/min), were found to contain less than 0·2% of the total activity in the plant. The apex counts have therefore not been included in the percentage distributions of ^{14}C given in Tables 1 and 2.

III. Results

(a) *Time of Translocation of the Long-day Stimulus*

Results presented in Figure 1 of an earlier paper (Evans 1960*b*) indicated that the long-day stimulus began to move out of the leaf blade just before the second high intensity light period, but moved out mainly during that period. The results of another experiment, given in Table 1 of the same paper, suggested a rather earlier movement of the stimulus out of the long-day leaf blade. In subsequent experiments, the results of which are summarized in Figure 2, the period of apparent translocation

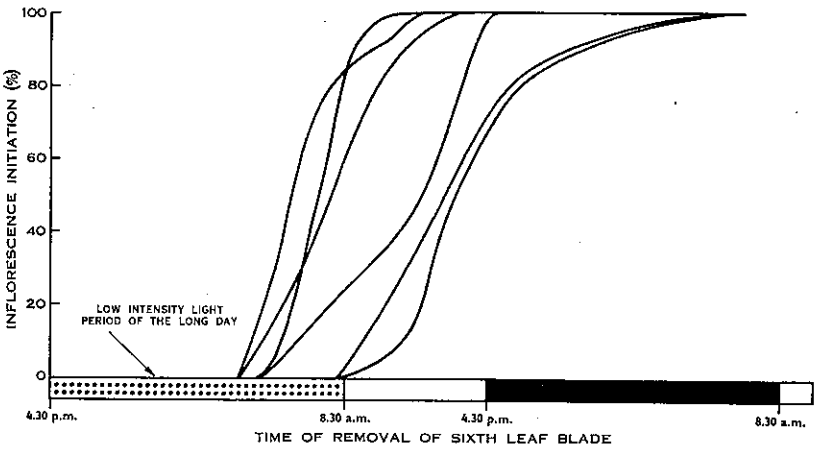

Fig. 2.—Effect of time of removal of the sixth leaf blade from plants exposed to one long day on percentage inflorescence initiation. Results are for six experiments in which no lower leaves were present.

of the long-day stimulus from the leaf blade has varied by about 8 hr. Differences were probably due to variations in age and seasonal light conditions, like those found by Salisbury with *Xanthium* (Liverman 1955). The apparent translocation curve for plants grown at the same time as those used in one of the labelling experiments is given in Figure 3, along with the results for exposure to one long photoperiod of various lengths.

For these plants the critical photoperiod was between 14 and 16 hr, although flowering response, in terms of the rate of apical development, increased with increase in the length of the continously lit period up to 32 hr. The results of removal of the sixth leaf blade at various intervals indicate that sufficient long-day stimulus to induce subsequent inflorescence development has moved out of the leaf blade 4 hr after the criticial photoperiod was reached. The apex length data suggest that

translocation of the stimulus from the leaf blade continued throughout the succeeding daylight period.

(*b*) *Influence of Lower Leaves on Translocation from the Sixth Leaf to the Shoot Apex*

A summary of the results of the first labelling experiment is given in Table 1. The apex counts clearly indicate that there is no reduction in the movement of assimilates from the sixth leaf to the shoot apex when the lower leaves are present. If anything, there is a slightly greater movement to the apex in their presence, as was found by Thrower (1962) with soybeans. In our experiments this may be due to the slightly higher fixation of $^{14}CO_2$ by the sixth leaf in the presence of the lower leaves, and the apex counts for the two treatments are even closer than those given in the table when corrected for differences in the total $^{14}CO_2$ fixation.

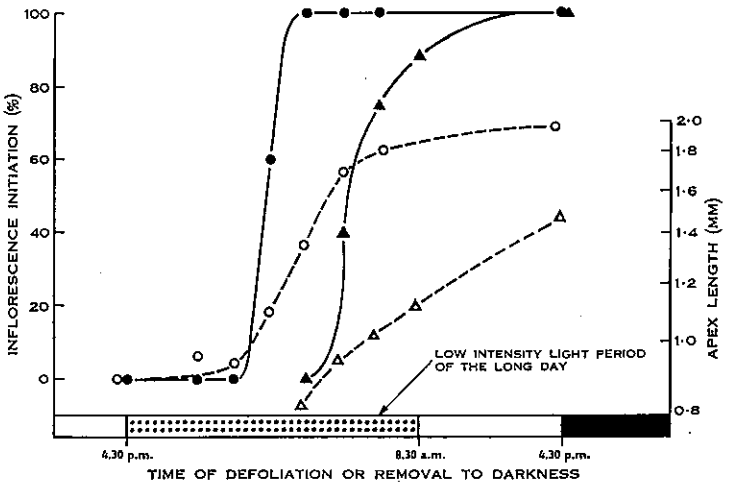

Fig. 3.—Effect of photoperiod length during a single long-day exposure, and of time of removal of the sixth leaf blade, on percentage inflorescence initiation (———) and on apex length (– – – –) at dissection, 3 weeks after the long-day exposure. ●,○ Plants removed to darkness at the times indicated. ▲,△ Sixth leaf blade cut off at the times indicated.

That the older leaves in no way act as a sink for assimilates from the sixth leaf is clear from the extremely small proportion of ^{14}C found in them. When these leaves are present, the sixth leaf supplies much less assimilate to the roots.

The results of a third labelling experiment support all these conclusions. Not more than 2% of the $^{14}CO_2$ assimilated by the sixth leaf was translocated to the lower leaves, including their leaf sheaths, and in the presence of these leaves less assimilate from the sixth leaf moved into the roots, and slightly more reached the shoot apex.

(*c*) *Distribution of Assimilates from the Fourth and Sixth Leaves*

The results of the second labelling experiment are given in Table 2. It is clear from these that the total fixation of $^{14}CO_2$ by the fourth leaf is less than half that by the sixth leaf. This is due partly to its smaller area, but also to a significantly lower

($P < 0{\cdot}001$) photosynthetic rate, measured as total ^{14}C per plant in counts per minute per unit leaf area. Of the ^{14}C assimilated by the fourth leaf, a greater proportion remained in the leaf, and a far greater proportion was supplied to the roots, than was

TABLE 1

EFFECT OF THE PRESENCE OF LOWER LEAVES ON TRANSLOCATION OF ^{14}C-LABELLED ASSIMILATE FROM THE SIXTH LEAF DURING 7 HR OF HIGH INTENSITY LIGHT (3500 F.C.) AT 25°C

Apex counts are at infinite thinness, all others at infinite thickness: all counts given on the basis that the whole of the treated leaf blade was exposed to $^{14}CO_2$. 16 replicates per treatment

Plant Part Measured	Relative Total Activity (counts/min)			Percentage Distribution of ^{14}C*		
	No Lower Leaves	Lower Leaves Present	Significance of Difference†	No Lower Leaves	Lower Leaves Present	Significance of Difference†
Treated leaf	19,258	21,834	$P < 0{\cdot}05$	39·9	42·6	n.s.
Lower leaves	—	267	—	—	0·6	—
Rest of shoot	26,579	29,756	$P < 0{\cdot}05$	53·4	53·7	n.s.
Roots	2,758	1,256	$P < 0{\cdot}001$	6·7	3·1	$P < 0{\cdot}001$
Apex	642	790	n.s.			
Total (excluding apex)	48,595	53,113	n.s.			

* Apex counts excluded. † n.s., not significant.

the case for the sixth leaf. On the other hand, the fourth leaf provided only about one-sixth as much assimilate to the shoot apex as did the sixth leaf. Only a very small proportion of assimilate from either leaf was found in the fifth leaf.

TABLE 2

COMPARISON OF THE DISTRIBUTION OF ^{14}C-LABELLED ASSIMILATE FROM THE FOURTH AND SIXTH LEAVES DURING 7 HR AT 25°C UNDER HIGH LIGHT INTENSITY

Apex counts are at infinite thinness, all others at infinite thickness: all counts given on the basis that the whole of the treated blade was exposed to $^{14}CO_2$. 16 replicates per treatment

Plant Part Measured	Relative Total Activity (counts/min)		Percentage Distribution of ^{14}C*		
	Sixth Leaf	Fourth Leaf	Sixth Leaf	Fourth Leaf	Significance of Difference
Treated leaf	20,550	10,339	39·8	48·3	$P < 0{\cdot}05$
Fifth leaf	150	209	0·30	0·94	$P < 0{\cdot}01$
Rest of shoot	28,746	7,999	55·4	32·2	$P < 0{\cdot}001$
Roots	2,313	4,678	4·5	18·9	$P < 0{\cdot}001$
Apex	710	129			
Total (excluding apex)	51,759	24,733			

* Apex counts excluded.

Again, the results of the third labelling experiment support all these conclusions. The fourth leaf provided only about one-sixth as much assimilate to the shoot apex

as did the sixth leaf, but a far higher proportion of that to the roots. A higher proportion of the fixed $^{14}CO_2$ was translocated to the older leaf blades and sheaths from the fourth leaf than from the sixth, but the proportion was extremely low in all cases.

IV. Discussion

(*a*) *The Inhibitory Role of Short-day Leaves*

It has previously been shown that lower leaves held in short-day conditions have a pronounced inhibitory effect on the inflorescence induction which follows exposure of an upper leaf to one long day. This inhibitory effect is greater the longer the lower leaves are left on the plants, and the greater their total area (Evans 1960*b*). It could be due:

(1) to the lower short-day leaves acting as sinks for the inductive stimulus translocated from the upper long-day leaf;

(2) to the lower leaves generating a solute stream which prevents the inductive stimulus from reaching the shoot apex;

(3) to dilution of the stimulus reaching the apex by assimilates from the lower leaves;

(4) to the production of a transmissible inhibitor of induction by leaves in short days.

The last interpretation is the one previously favoured but, in view of Zeevaart's (1962, 1963) scepticism, the likelihood of the first three is reconsidered. As noted above, direct evidence bearing on (1) and (2) cannot be obtained since the identity of the inductive stimulus is unknown. It is widely assumed, however, that this stimulus moves in the phloem in a pattern very similar to that for movement of assimilates (Lang 1952). Support for this view comes from the work on *Perilla* by Chailahjan and Butenko (1957), in which there was a striking parallelism between the movement of labelled assimilates and the apparent movement of the inductive stimulus over a wide array of leaf and receptor bud arrangements. Further support comes from the work of Lincoln, Raven, and Hamner (1956) with variously shaded receptor and donor branches in *Xanthium*. Possibilities (1) and (2) above will therefore be considered in terms of the pattern of assimilate translocation.

The results of the labelling experiments reported here indicate clearly that only a very small proportion, about 1%, of the assimilates from the upper long-day leaf move to the lower short-day leaves. These leaves, therefore, can hardly be regarded as effective sinks.

Nor do they reduce translocation from the upper long-day leaf to the shoot apex. If anything, translocation from the sixth leaf to the apex was slightly greater in the presence of the lower leaves.

The results of the labelling experiments also show that the fourth leaf supplies only about one-sixth as much assimilate to the shoot apex as does the sixth leaf. The much smaller, older, and more shaded leaves below the fourth are likely to provide far less assimilate than the fourth to the apical sink, as in the soybean (Thrower 1962). Yet the fourth leaf and those below it may have a marked inhibitory effect on induction when held in short days. Moreover, lower leaves in long-day conditions would

provide at least as much assimilate to the apex as those in short days, but there is no evidence from earlier experiments that their presence reduces the flowering response. In fact, it slightly increases the response. In one experiment, mean apex length at dissection was 1·08 mm when only the fifth leaf and 1·14 mm when all leaves were present during long-day induction. In another, apex length was 1·96 mm when only the sixth leaf and 2·36 mm when all leaves were present during the long-day exposure. Dilution of the inductive stimulus reaching the apex by assimilates from the lower leaves therefore seems unlikely to be of sufficient extent to account for the inhibitory effect of lower leaves in short days, and we conclude that this is more probably due to their production of a transmissible inhibitor of induction.

(*b*) *Estimation of the Rate of Translocation of the Long-day Stimulus*

Few estimates have been made of the rate of translocation of the floral stimulus, and all have yielded rates well below the usual rates for carbohydrate movement. Chailahjan (1940) found a rate of less than 1 mm/hr in *Perilla* plants, under rather unfavourable conditions. By ingenious use of two-branched plants of *Pharbitis*, with varying distances between donor leaf and receptor bud, Imamura and Takimoto (1955) obtained a rate of 2·6–3·8 mm/hr. This estimate assumed that upward and downward movements were at the same rate, whereas there was some evidence that upward movement was more rapid. Moreover, differences between their translocation and time-measuring groups of plants may have led to an underestimation of the rate. In a subsequent study (Imamura and Takimoto 1956) the transmission rate of the stimulus across a graft union was found to be 2·4–2·8 mm/hr. Zeevaart (1962) estimated a rate of 3·5 mm/hr for movement from cotyledons of *Pharbitis*, but this is bound to be an underestimate as it was assumed that the stimulus had to move across the petiole only, whereas some would also have to traverse the cotyledon.

The main problem is that, because a threshold amount of the floral stimulus must move before any response, which can only be measured by subsequent morphological development, can be recorded, the rate of translocation tends to be underestimated. The results of an earlier experiment (Evans 1960*a*, Fig. 4) help us deal with this problem of a threshold. Various areas of the sixth leaf blade were exposed to one long day, and it was found that 1·5 cm^2 yielded no flowering response, 3 cm^2 a threshold response, 5 cm^2 gave inflorescence initiation in almost all, and 6 cm^2 in all plants. Thus, to get initiation in all plants all the long-day stimulus generated by the basal 6 cm^2 of the leaf blade would have to move into the leaf sheath before removal of the blade. As the length of the blade in the basal 6 cm^2 was 8 cm, some of the floral stimulus must move this distance prior to defoliation for initiation to occur.

The plants used in this earlier experiment were almost identical in age, form, leaf size, and long-day response with those used in the experiment for which the results are given in Figure 3 above. In this experiment it was found that 4 hr must elapse before leaf-blade removal if a flowering response equivalent to that of the critical photoperiod of 16 hr was to be obtained.

These results suggest that some of the long-day stimulus must move at least 8 cm in 4 hr, at a rate of 2 cm/hr. This rate is likely to be an underestimate, because

it neglects any processes which may need to be consummated in the leaf after the critical photoperiod is reached, and because it assumes that all the stimulus from the basal part of the leaf has moved out in 4 hr. However, the estimate does bring the rate of movement of the floral stimulus within the range of estimated rates of carbohydrate translocation (Canny 1961).

V. Acknowledgments

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