INFLORESCENCE INITIATION IN *LOLIUM TEMULENTUM* L.

III. THE EFFECT OF ANAEROBIC CONDITIONS DURING PHOTOPERIODIC INDUCTION

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[*Manuscript received October* 16, 1961]

Summary

The aim of the experiments reported here was to find some condition other than light which could selectively control the operation of the inhibitory and promotive photoperiodic processes in the long-day plant *L. temulentum*, and hence allow a resolution of the photoperiod requirements of these processes.

Whole plants held continuously in aerobic short days showed no inflorescence initiation, whereas about two-thirds of those held in nitrogen for one long night subsequently initiated inflorescences.

Anaerobic conditions did not affect the ability of leaves in long days to stimulate inflorescence initiation. Leaves held in air throughout a 16-hr dark period had an inhibitory effect on inflorescence initiation and development, but those held in nitrogen throughout the dark period did not.

It is concluded that the promotive process occurring in the leaves of *L. temulentum* is unaffected by anaerobic conditions and accelerated by long days, while the inhibitory process in short-day leaves apparently cannot occur in the absence of oxygen.

I. Introduction

In the preceding paper of this series (Evans 1960*b*) it was concluded that leaves of *L. temulentum* held in short days have a net inhibitory effect on inflorescence initiation, while leaves in long days have a net promotive effect. This does not necessarily mean that long-day conditions are required for the promotive process to occur. It is also possible that the promotive process in the leaves occurs independently of day length, but that a net promotive effect is evident only in long-day leaves where the inhibitory process is reduced or absent.

To determine whether long days are required by the promotive process we need a condition other than day length which prevents the inhibitory process from occurring, but does not affect the promotive process. Low temperatures retard both processes (Evans 1960*b*) while high temperatures probably retard the promotive process before they affect the inhibitory one.

Earlier work on the flowering of long-day plants in short days, where the plants were held in an atmosphere of nitrogen during the dark period, suggested that anaerobic conditions might provide a suitable selective condition. Melchers and Claes (1943) induced flowering in annual *Hyoscyamus niger*, kept in 10-hr photoperiods, by holding the plants in nitrogen for the first 5 or 8 hr of the daily dark periods for 22–45 days. With *Rudbeckia bicolor*, another long-day plant, Zhdanova (1950) was able to induce bolting in 10-hr photoperiods by holding the

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plants in nitrogen for the whole or part of each dark period in 30–40 days. This was confirmed by Chailahjan and Konstantinova (1960), who also found an acceleration of flowering in *Avena sativa* by similar treatment.

The results given below show that *L. temulentum* plants will initiate inflorescences in short days after exposure to only one anaerobic dark period, and that this appears to be due to suppression of the dark inhibitory process in short-day leaves.

II. Experimental Methods

Most of the materials and conditions for the experiments reported here have already been described (Evans 1960*a*). All plants were grown for at least 5 weeks in 8-hr days until the sixth leaf was fully expanded.

The plants were given the various light and nitrogen treatments to be described only at the end of a day of bright sunshine. After treatment they were returned to the standard short-day conditions (8-hr photoperiods at 25°C/20°C) for 3 weeks before dissection of the apices of the primary shoots. Plants were recorded as having initiated inflorescences when they had at least reached the double ridges stage of differentiation. In some of the treatments where the bases of the plants were held in nitrogen for 16 hr, the apices of the primary shoots of a few plants subsequently died. These plants were omitted from the estimations of mean apex length and percentage initiation given in Tables 1–4.

In some treatments anaerobic conditions were applied only to individual leaf blades, or to the base of the plants. In others, not only atmospheric composition but also day length differed from one leaf to another. For these treatments air-tight wooden boxes, glazed at the top and made in two parts, were used, these being held in a temperature-controlled, artificially lit cabinet. The temperature was kept at 20°C throughout each 16-hr treatment period. Darkness inside the boxes was obtained by covering the glazed top with four layers of black cloth. The top of the lower part of each box was level with the junction between the fifth leaf blades of the experimental plants and their leaf sheaths. The upper, glazed part of each box sat directly above the lower one, the edges of both sections being continuously gasketed with opaque rubber tubing. Leaves were passed between the two gaskets, and the two sections of each box were then clamped together by four spring clips.

Air or nitrogen was admitted to the boxes, along one side of the upper sections, through light-trapped copper tubes, and was exhausted through similar tubes on the other side of the lower sections. When the relevant leaves or plants were enclosed, the boxes were immediately flushed out with commercial oxygen-free nitrogen at a high flow rate. After several minutes the flow was reduced to about 500 ml/min for the rest of the 16-hr period, giving a renewal of the atmosphere in the box once every 30 min. The oxygen concentration in the boxes was not measured but, in the absence of plants, no CO_2 could be detected in them by an infrared gas analyser after nitrogen had been passed through for 20 min, by which time displacement of the air in boxes by nitrogen was presumably almost complete.

In all experiments the aerobic treatments were set up in exactly the same way, but air was pumped into the boxes at about 500 ml/min.

III. Results

(*a*) *Effect of Anaerobic Conditions on Plants Held in Short Days*

In four experiments, groups of plants were held continuously in short days, but spent one long night in nitrogen. In the first three experiments all leaves were left on the plants, which were totally enclosed in the boxes (Fig. 1(*a*)). In the fourth

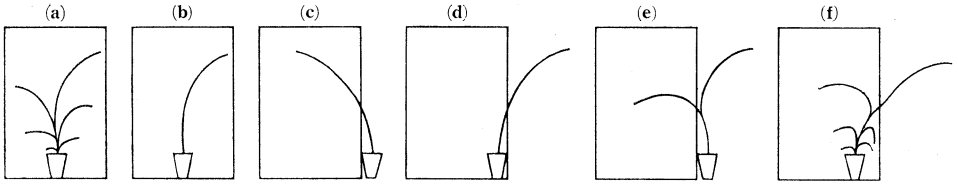

Fig. 1.—(*a*)–(*f*) Arrangement of plants in the various experiments. The boxes were filled with either air or nitrogen, and plants in them could be under either long- or short-day conditions.

experiment, all leaves except the sixth were removed just before treatment, and either the whole plant (Fig. 1(*b*)), or only the sixth leaf blade (Fig. 1(*c*)), was enclosed in nitrogen. Besides the short-day controls, which were held in boxes with air pumped through them, groups of plants were exposed to 16 hr of low-intensity illumination,

Table 1

EFFECT OF ANAEROBIC CONDITIONS DURING ONE 16-HR DARK PERIOD ON THE PERCENTAGE NUMBER OF PLANTS INITIATING INFLORESCENCES AND ON APEX LENGTH IN PLANTS HELD CONTINUOUSLY IN SHORT DAYS

Treatment	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Apex Length (mm)	No. of Plants (%)	Apex Length (mm)	No. of Plants (%)	Apex Length (mm)	No. of Plants (%)	Apex Length (mm)	No. of Plants (%)
Plants continuously in short days:								
Whole plant in air	0·77	0	0·84	0	0·86	0	0·84	0
Whole plant in nitrogen	0·78	67	0·92	60	0·88	63	0·92	70
Blade of 6th leaf in nitrogen							0·88	50
Plants given 1 long day	2·41	100	1·91	100	2·41	100	2·86	100

following 8 hr in daylight, as long-day controls. There were 8–12 plants per treatment, and the results are given in Table 1. In the first experiment the apex of the primary shoot died in 3 of the 12 nitrogen-treated plants, and in the second 2 of 10 died. None died in the remaining two experiments.

While none of the plants held continuously in aerobic short days initiated inflorescences, in each experiment about two-thirds of those which had one dark period in nitrogen did so. In the treatment in the fourth experiment, where only the sixth leaf blade was in nitrogen, 50% of the plants also initiated inflorescences. Zhdanova (1950) obtained bolting in *Rudbeckia* in short days only when the plants were in anaerobic dark periods for more than 30 days. Here, in *L. temulentum*, anaerobic conditions for only one night have led to the differentiation of double ridges—the usual criterion for inflorescence initiation in the grasses—in a substantial proportion of the plants. But whereas such initiation after long-day treatment is usually accompanied by a marked increase in the length of the apices (as shown

TABLE 2

EFFECT OF ANAEROBIC CONDITIONS DURING 16 HR OF LOW-INTENSITY ILLUMINATION ON THE EFFECTIVENESS OF LONG-DAY EXPOSURES

Treatment	Mean Apex Length (mm)	Inflorescence Initiation (%)
Experiment 1		
Short-day controls	0·82	0
Long day: base in air, leaf blades removed	0·88	0
Long day: base in air, leaf blade in air	1·26	100
Long day: base in air, leaf blade in nitrogen	1·30	100
Experiment 2		
Short-day controls	0·86	0
Long day: whole plant in air	1·99 *	100
Long day: whole plant in nitrogen	1·60 *	100
Experiment 3		
Short-day controls	0·83	0
Long-day controls: not enclosed	1·35	100
Long day: base in air, leaf blade in air	1·35	100
Long day: base in nitrogen, leaf blade in air	1·21	100

* Difference significant at $P<0·02$.

in the bottom line of Table 1), and in the rate of morphological differentiation, it is striking that no such increase has occurred in the nitrogen-treated plants, apex length being substantially the same in all plants held in short days regardless of whether or not they initiated inflorescences.

(*b*) *Effect of Anaerobic Conditions on the Promotive Effect of Long-day Leaves*

After 8 hr in air under daylight, plants were exposed to nitrogen throughout one 16-hr period of incandescent illumination of 40 f.c. intensity. All leaves except the sixth were removed from all plants at the end of the daylight period. The nitrogen treatments were given in three different ways, and the results for all experiments are given in Table 2. There were 10–13 plants in each treatment.

In the first experiment only the leaf blades were enclosed in the boxes, the base of all plants (i.e. the leaf sheaths, the enclosed shoot apex and stem, and the roots) being in air (Fig. 1(*c*)). That the base did not contribute significantly to the long-day response may be seen from the fact that there was no inflorescence initiation in the plants exposed to the long day, but with all leaf blades removed. The effectiveness of the leaf blades exposed to the long day in initiating inflorescence development was apparently unaffected by anaerobic conditions.

In the second experiment the plants were wholly enclosed in the boxes throughout the 16-hr period of low-intensity illumination (Fig. 1(*b*)). In this situation, anaerobic conditions did not prevent initiation in any plant, but significantly reduced the rate of inflorescence development. At dissection none of the nitrogen-treated plants in this experiment had a dead apex on the main stem. This result is comparable to those obtained by Chailahjan and Konstantinova (1960) with plants of *Rudbeckia bicolor* and *Avena sativa* held in nitrogen during the supplementary light period. This treatment, lasting 15 days, delayed flowering in the oats and prevented it in *Rudbeckia*.

In the third experiment, the leaf blades were in air and only the base of each plant was enclosed in the boxes (Fig. 1(*d*)). Here, the rate of inflorescence development was reduced by holding the base of the plants in nitrogen, though not significantly. In 3 of the 13 plants held in nitrogen the apex of the primary shoot subsequently died. The identical results for the two groups of wholly aerobic long-day plants in the third experiment—one partly enclosed in a box, the other standing free—indicate that having the leaf blade under pressure where it passes through the gasket has not reduced the response of the plants to the long-day exposure.

(*c*) *Effect of Anaerobic Conditions on the Inhibitory Effect of Short-day Leaves*

To avoid the adverse effects of anaerobic conditions on the shoot apex, only the short-day leaf blades should be enclosed in nitrogen, the long-day leaf and the base of each plant remaining in air. But it is then very difficult, with the present equipment, to have more than one leaf blade on each plant under anaerobic short-day conditions inside the boxes. This means that under aerobic conditions the observable inhibitory effect of the single short-day leaf will be relatively slight. It has previously been shown (Evans 1960*b*) that the net inhibitory effect of short-day leaves increases with increase in their total area. It also varies markedly from one experiment to another—as does the promotive effect of long-day leaves—in association with variations in the tillering habit of the plants, and presumably due to variations in the intensity and spectral composition of daylight. Such variations may be seen in the results of five experiments given in Table 3.

In all these experiments the sixth leaf blade and the base of each plant was in air under incandescent illumination of 40 f.c. intensity for 16 hr, while the fifth leaf blade was enclosed in a box in darkness for the same period, in either air or nitrogen (Fig. 1(*e*)). In the long-day control groups all leaves other than the sixth were removed at 4 p.m. prior to the period of low-intensity illumination, while in the other groups all leaves other than the fifth and sixth were removed at that time,

and the short-day (fifth) leaf blades were removed at 4 p.m. the following day, by which time most of their inhibitory effect should have been translocated from them (Evans 1960*b*).

In all experiments the short-day plants remained vegetative and all others initiated inflorescences. In these experiments the inhibitory effect of having the fifth leaf in aerobic short-day conditions during long-day induction is apparent only in the reduced apical development. One reduction is significant at the 5% level, and three others just fail to reach this level, but the consistency of the

TABLE 3

EFFECT OF ANAEROBIC CONDITIONS DURING ONE 16-HR DARK PERIOD ON THE INHIBITORY EFFECT OF ONE SHORT-DAY LEAF PRESENT DURING EXPOSURE OF THE SIXTH LEAF TO ONE LONG DAY

10–14 plants per treatment. All plants other than the short-day controls initiated inflorescences

Treatment	Experiment No.				
	1	2	3	4	5
	Apex Length (mm)				
Short-day controls	0·84	0·84	0·84	0·97	0·82
Long-day controls: sixth leaf only	2·86	1·96 *	1·87	2·87	1·42
Sixth leaf in long day, fifth leaf in short day in air	2·57	1·76	1·70	2·66	1·31
Sixth leaf in long day, fifth leaf in short day in nitrogen	2·91	1·94	2·01	2·79	1·46
	Leaf Area (cm^2)				
Sixth leaf	19·0	15·0†	12·0†	20·9	19·1
Fifth leaf	16·4	16·0	17·4	17·1	11·5

* Difference significant at $P<0·05$.

† Area reduced by cutting off part of the sixth leaf.

reduction in all five experiments suggests it is real. On the other hand, the presence of short-day leaves under anaerobic conditions has caused no reduction in the rate of apical development—except possibly in experiment 4—and has even yielded slight increases.

Greater inhibitory effects due to the presence of short-day leaves were obtained in two experiments where all leaves except the sixth were held in short-day conditions while the sixth was exposed to one long day (Fig. 1(*f*)). In these treatments the base of each plant was enclosed in the boxes under either aerobic

or anaerobic short-day conditions, with only the sixth leaf blade in air and under low-intensity illumination. All leaves except the sixth were then removed at 4 p.m. the following day. The long-day control plants in these experiments had all lower leaves removed at 4 p.m. before the period of low-intensity illumination, and their bases were enclosed in the boxes in short-day conditions, in air or nitrogen as required. The results of these experiments are given in Table 4.

TABLE 4

EFFECT OF ANAEROBIC CONDITIONS DURING ONE 16-HR DARK PERIOD ON THE INHIBITORY EFFECT OF SHORT-DAY LEAVES PRESENT WHILE ONLY THE SIXTH LEAF BLADE WAS EXPOSED TO ONE LONG DAY IN AIR

9–13 plants per treatment

Treatment	Experiment 1		Experiment 2	
	Apex Length (mm)	Inflorescence Initiation (%)	Apex Length (mm)	Inflorescence Initiation (%)
Short-day controls	0·86	0	0·83	0
Sixth leaf in long day:				
Base of plant in air, in short day				
Defoliated	2·41 **	100	1·35 *	100
Lower leaves present	1·63	100	1·19	90
Base of plant in nitrogen, in short day				
Defoliated	1·60	100	1·21	100
Lower leaves present	1·53	100	1·25	100
	Leaf Area (cm^2)			
Sixth leaf	12·0		15·5	
Fifth leaf	32·2		26·2	

* Difference significant at $P<0·05$.

** Difference significant at $P<0·01$.

As has already been shown, enclosure of the defoliated base of the plants in nitrogen reduces their response to exposure of the uppermost leaf to one long day. Thus, the inhibitory effect of short-day leaves held in nitrogen can only be assessed by comparing the results for defoliated and non-defoliated plants with their bases held in nitrogen, the lower pair of treatments given in Table 4. In these, the presence of the lower leaves in short days has had no net inhibitory effect. In the upper pair of treatments, however, with the plants in aerobic conditions, the

presence of the lower leaves in short-day conditions during exposure of the uppermost leaf to one long day significantly reduced the rate of inflorescence development in both experiments.

IV. Discussion

(*a*) *Selective Action of Anaerobic Conditions*

The evidence presented in Table 2 indicates that nitrogen does not reduce the net promotive effect on inflorescence initiation in *L. temulentum* of leaves in long days, while that in Tables 3 and 4 suggests that it eliminates the net inhibitory effect of short-day leaves. This effect is presumably due to the absence of oxygen, rather than of CO_2. Langston and Leopold (1954) found that removal of CO_2 during the dark period had no effect on flowering in barley in either short or long days. With *L. temulentum* removal of CO_2 from air passed into the boxes during 16-hr periods of either darkness or low-intensity illumination had no effect on subsequent flowering.

Anaerobic conditions could prevent translocation of the inhibitory effect from short-day leaves. However, Willenbrink (1956) found enclosure in atmospheres of nitrogen or hydrogen not to affect the movement of substances through the vascular bundles of *Pelargonium*, although a wide range of metabolic inhibitors did so. Presumably, the glycolysis of sucrose in vascular bundles (Turkina 1960) can provide energy for translocation under anaerobic conditions. In any case translocation of the inhibitory effect largely occurs in the subsequent period of high-intensity illumination (Evans 1960*b*) by which time the plants were returned to air.

Anaerobic conditions might also be envisaged as preventing the shoot apex from subsequently responding to the inhibitory effect translocated from short-day leaves. But this would not account for the suppression of the net inhibitory effect of short-day leaves when only the leaf blades were enclosed in nitrogen (Table 3), or for inflorescence initiation in plants held in short days when only the leaf blade was enclosed in nitrogen for one night (Table 1, expt. 4).

It seems likely, then, that there is an oxidative step in the generation of the inhibitory effect in leaves in long dark periods. Melchers and Claes (1943) identified the inhibitory effect of long dark periods on flowering in *Hyoscyamus niger* with respiratory consumption of carbohydrate. But CO_2 output may continue at quite high levels when the plants are held in nitrogen. Claes (1947) found the anaerobic CO_2 output to be about 60% of that for *Hyoscyamus* plants in air. In *L. temulentum* the anaerobic CO_2 output was found to be about 32% of the aerobic rate throughout the dark period. A change in the respiratory pathway may well be involved in induction, as Chailahjan and Aksenova (1960) have found a marked rise in the proportion of respiration inhibited by azide during photoperiodic induction in several long-day plants. But it remains an open question whether anaerobic conditions affect the dark inhibition through respiratory processes or through some other oxidative reaction.

The results of Ikuma and Thimann (1961) suggest that photoreversal of the red–far red pigment system in lettuce seeds is unaffected in nitrogen, but that photoreversal is closely followed by an oxidative step.

(*b*) *Requirement for Long Days*

Turning now to the question of whether long days are required for the occurrence of the promotive process in leaves of *L. temulentum*, a problem is posed by the criterion to be used for inflorescence initiation. The appearance of spikelet primordia, in the double ridges stage of differentiation, has been widely used as the criterion in work with grasses, and has been used throughout this series of papers. With this criterion, the results given in Table 1 indicate that long days are not required for inflorescence initiation when the dark inhibitory process in the leaves is suppressed in nitrogen. This is also the case for *Hyoscyamus niger* (Melchers and Claes 1943) and *Rudbeckia bicolor* (Zhdanova 1950).

But the results in Table 1 also indicate that suppression of the dark inhibition in nitrogen, although it may result in inflorescence initiation, does not lead to the increased rate of apical development characteristic of exposure to one long day. After one long anaerobic night the apices at dissection were 0·8–0·9 mm long, and about two-thirds of them had differentiated a number of spikelet primordia; after one long day, on the other hand, the apices were 1·9–2·9 mm long, all had differentiated lemma primordia, and floret primordia were evident in most. This difference could be due to incomplete suppression of the dark inhibitory process in anaerobic conditions, or it could be that long days, while not essential for inflorescence initiation in the absence of the inhibitory process, strongly accentuate the promotive process in the leaves of *L. temulentum*.

V. Acknowledgments

I would like to acknowledge the technical assistance of Mrs. Katie Bretz throughout these experiments, and the helpful scepticism of my colleagues Drs. L. A. T. Ballard and N. P. Kefford.

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