INFLORESCENCE INITIATION IN *LOLIUM TEMULENTUM* L.

V. THE ROLE OF AUXINS AND GIBBERELLINS

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Summary

Single applications of auxins and gibberellins were made at various times during long-day induction in the hope that determination of the site and time of their greatest effect on inflorescence induction might indicate which of the component steps in the flowering process are under their influence. Some problems in this approach are considered.

The auxins 3-indolylacetic acid and 1-naphthylacetic acid had their greatest effect when applied to the leaves. For plants given one long day auxins were significantly inhibitory when applied near the end of the daylight period, but had little effect at other times. On the other hand, auxins stimulated inflorescence initiation in plants held in short days but given a 2-hr exposure to light in the middle of one night, although alone neither the light break nor the auxin had any effect on initiation.

Of six gibberellins, gibberellic acid (GA_3) had the greatest effect. Single injections of 3 μg of GA_3 into plants held continuously in short days led to initiation in about half of the plants, with maximum effectiveness for injections at the end of the daylight period. Holding the leaves in an atmosphere of nitrogen during one long dark period following injection decreased the effect of gibberellin.

Applications of GA_3 to plants given one long day showed a marked cycle of effectiveness, this being greatest at the end of the daylight period of the long day, and least at the beginning and at the end of the long day. The antigibberellins (4-hydroxy-5-i-propyl-2-methylphenyl)-trimethylammonium chloride, 1-piperidine carboxylate (Amo 1618) and 2-chloroethyl trimethylammonium chloride (chlorocholine chloride) were without effect when applied to leaves, even on plants treated with gibberellin.

It is concluded that auxins probably influence induction in *L. temulentum* mainly through an effect on the long-day promotive process, while gibberellins most likely act by potentiating the shoot apex for induction, and also by reducing the effectiveness of the dark inhibitory process.

I. INTRODUCTION

The processes leading to the induction of flowering remain unknown. Extraction of the floral stimulus would provide the most direct clue for their elucidation, but such extracts have not yet been purified to any great extent (Lincoln, Mayfield, and Cunningham 1961). Analysis of induced leaves and shoot apices might also be expected to provide some clues, but quantitative and qualitative changes have been found in so many compounds that it is difficult to decide which are causes and which are effects of induction.

In this situation a profitable approach may be to treat plants with growth substances or specific antimetabolites, at a series of times during induction, in the

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hope that they may be effective only when applied at certain times, indicating which component processes of induction are under their influence. The rationale for this approach has been outlined by Salisbury (1961) and some problems in its interpretation will be considered later.

This paper deals with the effects of two groups of growth substances, the gibberellins and the auxins, both of which are known to affect flowering in long-day plants, on *Lolium temulentum* plants which require only one long day for induction. It has previously been shown that exposure of leaves to one long day leads to the production in them of a transmissible stimulus to inflorescence initiation. This moves out of the leaf blade most rapidly in the daylight period following the long day, but can begin to do so several hours earlier. Leaves in short days have a net inhibitory effect which also appears to be transmissible (Evans 1960*b*), and which can be eliminated by holding the leaves in an atmosphere of nitrogen during the dark period of the long day (Evans 1962). Inflorescence initiation will be affected by any substance influencing either the stimulatory or inhibitory processes in the leaves, the subsequent translocation of their products from leaf to shoot apex, or the ensuing apical processes which lead to induction. However, determination of the site and time of greatest effect should enable us to define which of these processes is affected by the applied substances.

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, before exposure to a long day or application of the growth substances. There were 10–16 plants per treatment. The long-day exposure consisted of 8 hr at 25°C under bright sunshine from 8.30 a.m. to 4.30 p.m., followed by 16 hr at 20°C under incandescent lamps which gave a light intensity of 15 f.c. at plant height. After treatment the plants 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. The stage of morphological development of each apex was recorded, together with its length above the base of the last overlapping leaf primordia. The close relation previously found between these parameters was not changed to any extent by the chemical treatments reported here.

In most experiments all leaves except the sixth were removed at the beginning of the long day, or prior to chemical treatment. Where the leaves were held in an atmosphere of nitrogen throughout a dark period the treatments were given as described in an earlier paper (Evans 1962). Manipulation of plants during the dark period was done under weak green light, which has been found to have no effect on inflorescence initiation in *L. temulentum*.

Various methods of applying the growth substances were tried, and some of the differences in effect between these will be described. For injections, 0·1 ml of the applied solution was injected by means of a hypodermic syringe into the cavity within the leaf sheaths and surrounding the growing point. Leaf blade applications

were made in three ways: as drops placed at the base of the sixth leaf blade; by immersing the sixth leaf blades in 30 ml of the solution for 10 min; or as a fine spray from an atomizer, using 2 ml per 10 plants. Control plants had distilled water applied to them in the same manner as the growth substances. Unless otherwise noted, wetting agents were not included in the treatment solutions. When used, Tween 20 was at a concentration of $0 \cdot 1\%$ both in treatment solutions and in Tween-only controls. In no case did Tween alone have any significant effect on flowering.

The auxins used were 3-indolylacetic acid (IAA) and 1-naphthylacetic acid (NAA) which were obtained from the California Corporation for Biochemical Research. Fresh solutions were prepared for each experiment, and were brought to neutrality by addition of $0 \cdot 1N$ KOH.

The six gibberellins used (GA_1, GA_3, GA_4, GA_5, GA_8, GA_9) were provided by Dr. P. W. Brian and Dr. J. MacMillan of Imperial Chemical Industries. Unless otherwise stated, treatments were made with gibberellic acid (GA_3). Of the two anti-
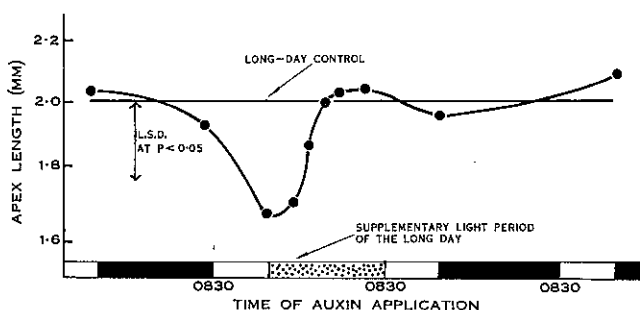

Fig. 1.—Effect of time of auxin treatment (IAA, 10^{-3}M) on inflorescence development in *L. temulentum* plants exposed to one long day. Auxin applied to the leaves, no Tween used.

gibberellins used, 2-chloroethyl trimethylammonium chloride (chlorocholine chloride or CCC) was supplied by the Cyanamid Co., and (4-hydroxy-5-i-propyl-2-methyl-phenyl)-trimethylammonium chloride, 1-piperidine carboxylate (Amo 1618) by the Rainbow Colour and Chemical Co.

III. RESULTS

(a) Effects of Auxin Applications

(i) *Applications during Long-day Induction.*—The first experiment with IAA, applied at various times during long-day induction, indicated that IAA was most inhibitory when applied between 4 p.m. and 9 p.m. during the single long-day exposure. An experiment was then carried out to examine, for applications at 8 p.m. on the long day, the effects of (1) the auxin (IAA or NAA) used, (2) its concentration, (3) the mode of application, and (4) the presence of Tween 20. The results need not be considered in detail, but the findings were as follows. IAA and NAA were equally inhibitory at equal molar concentrations, their inhibitory effect being significant only at concentrations above 3×10^{-4}M for leaf applications. With 10^{-3}M solutions,

injections were less effective than leaf applications, and of the various methods of leaf application, immersing the leaf blades was slightly more effective than spraying them, while drops applied at the base of the leaf blades were least effective. Tween 20 slightly increased the inhibitory effect of leaf applications, although Tween 20 alone had no effect.

The effect of time of auxin applications to leaves on flowering response can be seen in Figure 1, which gives the results of an experiment (No. 59) in which IAA was used, at a concentration of 10^{-3}M, without Tween. Clearly, auxin was inhibitory to induction only when applied in the latter half of the daylight period, or during the following 4 hr, of the one long day. This pattern of effect was evident in all six experiments, but the inhibitory effect was much more marked in some, completely preventing induction in one experiment (No. 20). The extent of inhibition can be gauged from Table 1, in which all entries for the 8.30 p.m. auxin applications differ significantly from the long-day controls.

TABLE 1

EFFECT OF AUXIN APPLICATIONS TO LEAVES AT 8.30 P.M. ON THE ONE LONG DAY ON INFLORESCENCE DEVELOPMENT IN L. TEMULENTUM PLANTS

All plants reduced to sixth leaf blade only at about 2 p.m. on the long day. Concentration of auxin solutions 10^{-3}M. Differences significant at $P < 0.05$ (*), $P < 0.02$ (**), and $P < 0.01$ (***) between long-day plants and long-day plants plus auxin are indicated

Expt. No.	Auxin Used	Tween Used	Apex Length (mm)		
			Short-day Plants	Long-day Plants	Long-day Plants + Auxin
20	IAA	—	0·96	1·72	0·96***
50	NAA	—	0·89	1·74	1·37**
52	NAA	—	0·79	3·51	2·44**
59	IAA	—	0·88	2·00	1·70*
62	IAA	+	0·91	2·08	1·59*
64	IAA	+	0·80	1·96	1·73*

(ii) *Applications to Plants Held in Short Days.*—Auxin applications can have a promotive effect on initiation when one long night is broken by light for 2 hr, a treatment which is ineffective on its own in causing initiation in *L. temulentum*. Two experiments of this kind have been carried out, one with IAA and one with NAA, but otherwise identical. The light break consisted of 2 hr of light from incandescent lamps, of 50 f.c. intensity at plant height, given in the middle of one long night. The results of the two experiments were very similar, and have been combined for presentation in Table 2(a). It may be seen that auxin applications alone, as either injections or sprays, had no effect on inflorescence initiation, nor had the light break of 2 hr alone. However, auxin applications combined with the light break led to inflorescence initiation in a proportion of the plants, this proportion being greater for the plants in which the auxin was applied as sprays to the leaves, and not markedly dependent on the concentration of auxin over a 40-fold range. The effect of time of application

of auxin in relation to the light break was also examined in the experiment with NAA, the results being given in Table 2(b). From these results it would seem that applications made during or after the light break may be slightly more effective than those made before it.

TABLE 2

Light break, consisting of light from incandescent lamps of 50 f.c. intensity at plant height, was for 2 hr in the middle of one long night (11.30 p.m.–1.30 a.m.) and was given when plants were 6 weeks old

(a) *Results of IAA and NAA experiments combined: auxin applied at 4 p.m.*

	Auxin Concentration (p.p.m.)					
	0	2·5	5	10	25	100
No light break	0	0	0	0	0	0
Light break						
Auxin injected	0	8	28	38	23	20
Auxin sprayed	0	—	50	—	42	40

(b) *NAA (25 p.p.m.) injected before, during, and after light break*

Time of NAA Injection	Inflorescence Initiation (%)	Time of NAA Injection	Inflorescence Initiation (%)
Before light break		After light break	
10 a.m.	10	10 a.m.	38
4 p.m.	17	4 p.m.	25
During light break			
Midnight	30		

(b) Effects of Gibberellin Applications

(i) *Methods of Application.*—Single applications of GA$_3$ by the methods described above, and at several concentrations, were made at 4.00 p.m. on the long day, at the end of the high intensity light period. The detailed results need not be given here, but the main findings were as follows. With all methods of application, GA$_3$ at a concentration of 3×10^{-4}M significantly increased the response to long-day exposure. In the presence of Tween 20 all methods of application were equally effective, but in its absence there was a tendency for the leaf applications to be less effective than injection. Single applications of GA$_3$ solutions at concentrations below 1×10^{-4}M had no significant effect on inflorescence development. In all the experiments described below gibberellin treatments were by injection, as the use of the wetting agent could then be avoided.

(ii) *Effectiveness of Various Gibberellins.*—Single injections of six gibberellins were given at 4.00 p.m. to two groups of plants, one held continuously in short days,

the other exposed to one long day at the time of injection. The results are given in Table 3. It is clear that there are marked differences in effectiveness among the gibberellins used here, and it is of interest that the order of effectiveness is identical in both the long- and short-day groups. GA_3 was the most effective, followed by GA_5 and GA_1. Response to the other gibberellins was not significant. Although this order of effectiveness is not identical with that for any other long-day plant, it is similar to that for *Centaurium minus* (Michniewicz and Lang 1962) and *Lactuca sativa* (Wittwer and Bukovac 1962). It differs from *Crepis parviflora* and *Bryophyllum crenatum* in the relative ineffectiveness of GA_4. GA_7, which is most active in the induction of flowering in those long-day plants examined to date (Michniewicz and Lang 1962), was unfortunately not available for trial.

TABLE 3

EFFECT OF VARIOUS GIBBERELLINS ON INFLORESCENCE INITIATION IN L. TEMULENTUM PLANTS

All gibberellins injected at 4 p.m. as 10^{-4}M solutions

Gibberellin Injected	Plants in Short Days		Plants Given One Long Day	
	Apex Length (mm)	Inflorescence Initiation (%)	Apex Length (mm)	Inflorescence Initiation (%)
None	0·92	0	2·87	100
GA_1	1·00	60	3·04	100
GA_3	1·45	100	3·67	100
GA_4	0·89	0	2·84	100
GA_5	1·23	70	3·11	100
GA_8	0·95	0	2·62	100
GA_9	0·95	0	2·84	100

(iii) *Effect of Antigibberellins.*—Salisbury (1961) has pointed out that time of application studies can be particularly informative when specific antimetabolites can be used. Two substances, CCC and Amo 1618, which have been shown to act like antigibberellins, were therefore tried. As Tolbert (1961) points out, these compounds are in no sense analogues of the gibberellins and likely to compete with them for specific reaction sites, but both have been shown to reverse the effects of applied gibberellins on a wide variety of plants. CCC was used in two experiments, in the first at a concentration of 10^{-2}M, being injected at a series of times into plants exposed to one long day. In this experiment it had no significant effect on the rate of inflorescence development at any time of application. In the second experiment, CCC and Amo 1618, in solutions of 10^{-3}M concentration, were injected at 4.00 p.m. into plants held either in short days or exposed to one long day at the time of treatment. The antigibberellin applications were made either alone, or immediately prior to injections of 10^{-3}M GA_3. The results of this experiment are given in Table 4. It can be seen that neither Amo 1618 nor CCC alone had any effect on either the percentage of inflorescence initiation or apex length, in either short- or long-day conditions. GA_3 had a highly significant effect in both conditions, and this was not significantly reduced by the simultaneous application of either CCC or Amo 1618. Thus, in *L. temulentum*,

with this method of application, there is no evidence that either of these compounds is acting as an antigibberellin. Margara (1962) has shown that a compound similar to CCC can antagonize gibberellin action only when it is applied several days before the gibberellin. It is also possible that the two compounds may be effective only when applied to the root medium of plants, as they usually are. Such restrictions would reduce their value in timing experiments, however, and for this reason no further experiments were carried out with them.

(iv) *Effect of Time of Gibberellin Application.*—Single injections of GA_3, in a solution of $10^{-4}M$ concentration, were given at four times during one day to plants held continuously in short days. The average percentage inflorescence initiation, in four experiments, for these treatment times are as follows: 9 a.m., 43%; 12.30 p.m., 50%; 4 p.m., 59%; 10.00 p.m., 27%. Applications at all times have elicited some flowering in non-inductive conditions, this being most marked for treatments at 4 p.m. All control plants remained vegetative. It is of interest that these responses are to single applications of about 3 μg of GA_3, which is much less than the amount usually required to cause flowering in long-day plants under non-inductive conditions.

TABLE 4

EFFECT OF ANTIGIBBERELLINS ON THE RESPONSE OF L. TEMULENTUM PLANTS TO GIBBERELLIC ACID (GA_3) INJECTIONS

All substances injected at 4 p.m. as $10^{-3}M$ solutions; n.s., differences not significant

Treatment of Plants Held in Short Days	Apex Length (mm)		Inflorescence Initiation (%)	Treatment of Plants Given One Long Day	Apex Length (mm)		Inflorescence Initiation (%)
Controls	1·08		0	Controls	3·47		100
+Amo 1618	1·18	n.s.	0	+Amo 1618	3·87	n.s.	100
+CCC	1·08		0	+CCC	3·74		100
+GA₃	2·06		100	+GA₃	5·75		100
+GA₃+CCC	1·85	n.s.	100	+GA₃+CCC	6·06	n.s.	100
+GA₃+Amo 1618	1·86		100	+GA₃+Amo 1618	5·72		100

Variation with time of application in the effect of single injections of GA_3 in plants exposed to one long day are given in Figure 2. Five experiments of this kind have been carried out, the results of the three largest experiments being given in the figure. The uppermost curve gives the results of an experiment in which the plants were 8 weeks old at the time of the long-day exposure, rather than the usual 5–6 weeks of age, hence the greater response to the long-day exposure. Although the time-dependence of the effect of GA_3 applications varies somewhat from one experiment to another, certain features are consistent in all cases. Thus, GA_3 always has its greatest effect when applied at the end of the daylight period of the one long day, and is only slightly less effective when applied at the same time on the following day. On the other hand, it has its minimum effect when applied at the beginning of the long day, about 7 hr before the time of maximum effect. In most experiments its effect at this time is not statistically significant. The other period of least effect is the second half of the long day. Thus, while applications at the beginning of the

supplementary light period of the long day have maximal effect, those given only 7 hr before or 7 hr after have a minimal effect. It is noteworthy that the time of maximum effect coincides with that for applications to plants held in short days, although the time-dependence of the effect is much more striking in plants given one long day.
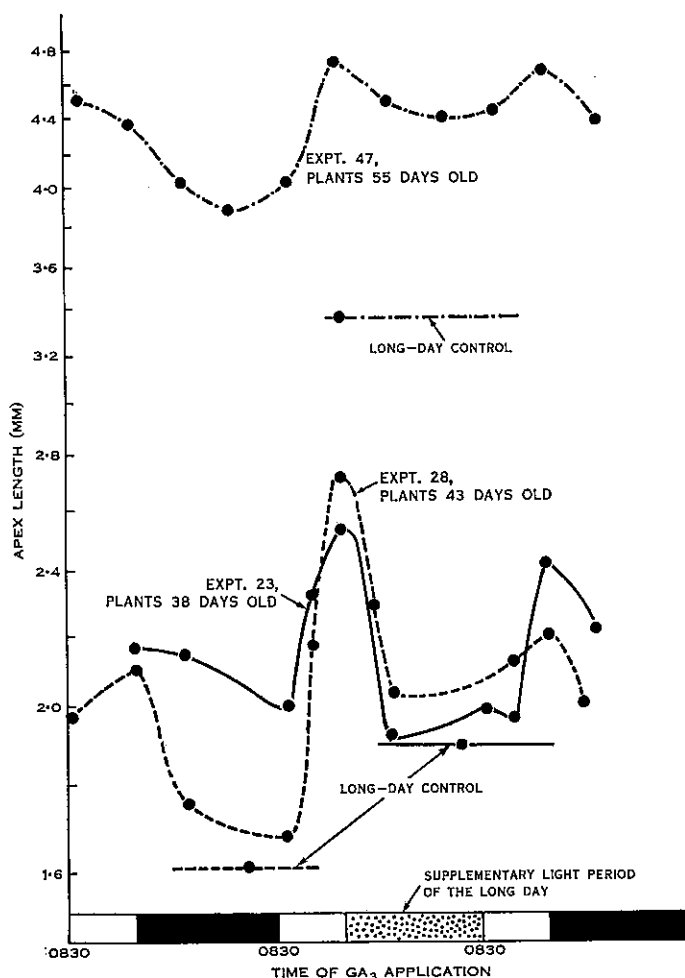

Fig. 2.—Effect of time of gibberellic acid treatment (10^{-3}M solutions, no Tween) on inflorescence development in *L. temulentum* plants exposed to one long day.

(v) *Effect of Anaerobic Conditions on the Response to GA_3.*—Since anaerobic conditions selectively prevent the expression of the inhibitory effect of short-day leaves in *L. temulentum*, three experiments were carried out to examine the effect of such conditions on the response to GA_3 injected just prior to the beginning of the anaerobic dark period. The results of these experiments are given in Table 5. As reported previously (Evans 1962), holding the leaves under anaerobic conditions for

one long dark period subsequently leads to inflorescence initiation in the majority of plants in each treatment group. However, although only the remaining leaf blade was under anaerobic conditions, while the GA_3 was injected near the apex under aerobic conditions, the nitrogen treatments have markedly reduced the response to GA_3 in all experiments.

TABLE 5

INTERACTION BETWEEN THE EFFECTS OF GIBBERELLIC ACID (GA_3) INJECTIONS, AND OF HOLDING THE ONE LEAF BLADE IN NITROGEN FOR ONE 16-HR DARK PERIOD, ON INFLORESCENCE INITIATION IN L. TEMULENTUM PLANTS HELD IN SHORT DAYS

All plants with leaf 6 only

Expt. No.		No GA_3		10^{-4}M GA_3 Injected at 4 p.m.	
		Leaf 6 in Air	Leaf 6 in Nitrogen	Leaf 6 in Air	Leaf 6 in Nitrogen
I	Apex length (mm)	1·07	1·12	1·61	1·38*
	Inflorescence initiation (%)	0	67	100	100
II	Apex length (mm)	0·90	—	1·12	0·97
	Inflorescence initiation (%)	0	—	90	25
III	Apex length (mm)	0·92	0·85	1·45	1·03*
	Inflorescence initiation (%)	0	50	100	27

* Difference between GA_3 treatments significant at $P < 0.05$.

IV. DISCUSSION

(a) Interpretation of Experiments on the Time of Effectiveness of Applied Compounds

The use of timing experiments, such as those reported above, in work on the induction of flowering has been outlined by Salisbury (1961). They are most logically carried out with plants which require exposure to only one inductive photocycle, and in which physiological analysis has established the site and time of occurrence of some of the component processes of induction, as in the short-day plants *Xanthium pensylvanicum* and *Pharbitis nil* which have been used for such experiments (Salisbury 1955, 1957; Salisbury and Bonner 1955, 1960; Bonner and Zeevaart 1962; Zeevaart 1962; Bonner, Heftmann, and Zeevaart 1963). Among long-day plants, *L. temulentum* has a sensitivity to photoperiodic induction which is comparable to that of *Xanthium* and *Pharbitis* among short-day plants, and the environmentally controlled component processes of induction in this species have been examined to some extent (Evans 1960a, 1960b, 1962).

Before discussing the results of the experiments reported here, some problems in deducing the role of a substance in induction from the time of its most effective application should be considered. The time-dependence of its effect will be determined not only by the timing of the component process affected by the applied substance but also by other factors.

The time required for uptake of the substance, and for its transport from the site of application to the site of action, will influence the timing of its effect, as shown schematically in Figure 3. Moreover, the rates of uptake and transport may also vary with the time of application. The site of action of applied compounds may sometimes be deduced from the relative effectiveness of leaf and tip applications, as with the 5-fluorouracil treatments in *Xanthium* (Salisbury and Bonner 1960; Bonner and Zeevaart 1962). In other cases, however, differences in the effectiveness of leaf and tip applications may reflect differences in relative uptake of the applied compound.

The rate of metabolism of the applied substance will also influence the time-dependence of its effect. If it is slowly metabolized, applications given well before the occurrence of the process controlled by the substance may still be effective, as shown in Figure 3.
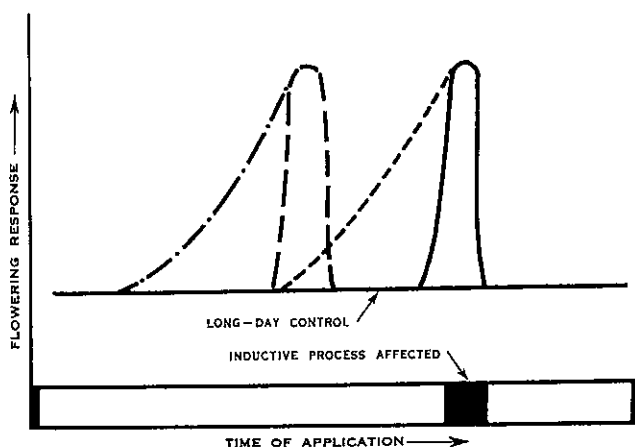

Fig. 3.—Schematic illustration of the time-dependence of the effect of an applied substance which specifically accelerates one component process of induction: ——— when rapidly taken up and rapidly metabolized, – – – – when rapidly taken up and slowly metabolized, —·—·— when slowly taken up and slowly metabolized, — ·· — ·· — when slowly taken up and rapidly metabolized.

Diurnal changes in the pool size or concentration of related endogenous substances may also vary the effect of applied substances. Where these occur, the applied substances may have a cycle of effectiveness more indicative of changes in endogenous levels than of the occurrence of specific processes controlled by them.

Some applied compounds may affect inflorescence induction in a relatively unspecific way, in which case one would not expect them to show a marked time-dependence of their effect for applications made during induction. Other compounds, such as some of those used by Salisbury (1957), may not influence induction but may modify subsequent inflorescence development. As effects on induction in the experiments reported here are determined 3 weeks after the long-day exposure, compounds affecting inflorescence development rather than induction could influence the stage of development at dissection, but only if they remained active in the plants for the 6 days following induction before morphological differentiation begins. In that event

they would hardly be expected to show any marked time-dependence of effect for applications made during induction.

(b) *Role of Auxins in Induction of* L. temulentum

Previous work on the effect of auxins on flowering in long-day plants has been reviewed by Lang (1961), who indicates the need for timing experiments, such as those reported here, for determination of their role in induction.

On the one hand, early work with *Circaea lutetiana* (Dostal and Hosek 1937) and *Calendula officinalis* and *Nemesia floribunda* (von Denffer and Gründler 1950) showed that auxin applications delayed flowering in plants under long days. On the other, work with *Hyoscyamus niger* and *Silene armeria* by Liverman and Lang (1956) showed that auxin treatments led to flower induction in plants grown in short days extended with light at an intensity which alone was insufficient to induce flowering.

Such opposite effects of auxin on flowering could have been due to different responses by different species. That this is unlikely is indicated by the results with *L. temulentum* in which induction was inhibited by auxin in plants exposed to one long day, but was promoted in plants kept in short days except for exposure to a light break of 2 hr during one night.

Chailahjan and Zdanova (1938) and Cooke (1954) have shown the endogenous auxin level to be higher in long days than in short ones in several long-day plants, and Lang (1961) has suggested that auxin applications to plants in long days raise the endogenous level to above-optimal concentrations, while those to plants in short days make the endogenous level more nearly optimal.

The timing experiments with plants exposed to one long day, and the greater effectiveness of leaf applications than of injections, suggest that a process sensitive to auxin levels occurs in the leaves in the hours immediately following the 8-hr period in daylight on the long day. It has been shown in Part IV of this series (Evans and Wardlaw 1964) that the long-day promotive process is consummated within 8 hr of the end of the daylight period. As shown in Figure 1, auxin applications are only inhibitory when applied within 6 hr of the end of the daylight period of the long day, which suggests that the long-day promotive process is sensitive to auxin level. The marked effect of auxin applications at this time for plants given one long day is unlikely to be due to more favourable conditions for uptake, since Thimann and Wardlaw (1963) found auxin uptake by green tissue to be particularly promoted by high intensity light. Nor is it likely to be merely a reflection of diurnal changes in endogenous auxin levels, since these are generally highest by day (Yin 1941; von Guttenberg and Kröpelin 1947; Kiyosawa 1960). It is possible, however, that diurnal changes in endogenous auxins follow a different course in long-day plants (Bezler and Bunning 1950).

The role of auxin in the inductive processes of plants held in short days but exposed to a light break of 2 hr during one long night is not clear. The most effective applications were those made during the daylight period following the light break, which at least suggests that the favourable effects of auxin were not due to effects on the promotive process in the leaves.

(c) *Role of Gibberellins in Induction of* L. temulentum

Applied gibberellins hasten or induce flowering in many long-day plants, particularly rosette ones, but usually only after repeated applications of substantial doses (Lang and Reinhard 1961). The induction of inflorescence initiation in *L. temulentum* in short days by single applications of only 3 μg of GA$_3$ indicates a greater sensitivity of this species to gibberellin action along with its greater sensitivity to long-day induction. This is in sharp contrast to the results of Peterson and Bendixen (1963) who found little effect on flowering of gibberellin solutions of extremely high concentration, when applied to seeds of a vernalizable strain of *L. temulentum*. It should be stressed that with plants of the age used in the experiments reported here, inflorescence initiation induced by GA$_3$ applications apparently follows the normal course of differentiation, unlike those so treated by Caso, Highkin, and Koller (1960).

Despite a great amount of work, the role of gibberellins in the induction of flowering in long-day plants remains obscure. The elegant grafting experiments of Harada (1962) and Zeevaart and Lang (1962) indicate that while gibberellin is not itself the floral stimulus, it is necessary for its production, but whether as precursor or cofactor is unknown.

Since no differences were found between leaf and tip applications of gibberellins in the extent or time of their effect, their site of action is not clearly indicated. This was to be expected in view of their rapid uptake and transport in plants (Sachs, Bretz, and Lang 1959). The very marked differences in effect of applications at 4 p.m. on the long day, and of those several hours earlier, also suggest rapid metabolism of applied gibberellins, at least during the day.

The timing of their effect makes it unlikely that gibberellins are influencing translocation of the stimulus from the leaves, or induction itself. That they may play a role in the photoperiodic processes in the leaves is suggested by the experiments with anaerobic leaf treatments. In these, holding the only remaining leaf blade in nitrogen during one 16-hr dark period greatly reduced the effect of gibberellin applications, even though these were by injection near the shoot apex, which was under aerobic conditions. This suggests that gibberellins participate in a step requiring aerobic conditions in one of the photoperiodic processes in the leaves. Since the long-day promotive process is unaffected by anaerobic conditions (Evans 1962), the gibberellin effect may be a suppression of the dark period inhibition. This would account for their induction of flowering in the plants held in short days, but not for their marked effect on plants undergoing a long-day exposure. Gibberellins may therefore play other roles in the day-length response.

The experiments of Sachs, Bretz, and Lang (1959) with *Hyoscyamus* and *Samolus* suggest that applied gibberellins act at the shoot tip, stimulating and phasing cell division in the subapical zone. One could envisage gibberellins potentiating induction at the shoot apex in *L. temulentum* by stimulating cell division there and by synchronizing it with the arrival of the promotive stimulus from leaves exposed to long days. This explanation could account for the greater effectiveness of gibberellins applied to plants exposed to one long day compared with those kept in short days. It could also account for the time-dependence of the effect of applications to plants

given one long day, since Sachs, Bretz, and Lang (1959) found the phased stimulus to apical cell divisions to continue for several days with a 24-hr periodicity, similar to that observed in their effect on induction in *L. temulentum*.

If gibberellins do play a role in more than one of the component processes of induction in long-day plants, it is possible that there may be a different order of effectiveness for the various gibberellins in the two processes. However, no difference was apparent in the order of effectiveness of those used here when applied to plants in short days, or given one long day.

V. ACKNOWLEDGMENTS

VI. REFERENCES

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