INFLORESCENCE INITIATION IN *LOLIUM TEMULENTUM* L.

XIII.* THE ROLE OF GIBBERELLINS

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[*Manuscript received January* 29, 1969]

Summary

Both stem growth and flowering in plants exposed to 1 long day showed an increasing response to gibberellins with increase in the concentration of the injected solution, up to 12×10^{-4}M. With plants in short days both responses were asymptotic or showed an optimum at 4×10^{-4}M, depending on the light intensity.

No synergism between GA_3, GA_4, and GA_7 was apparent.

2-Chloroethyltrimethylammonium chloride (CCC) had no effect on the flowering response of plants exposed to 1 long day, even at high concentration (0·1M) applied via the root medium at times varying from 3 days before to 6 days after the long day. Such applications caused marked reductions in growth, however.

When both CCC and GA_3 were applied, higher concentrations of CCC progressively reduced the stem growth response to applied GA_3. The flowering response was affected in the opposite manner, however, higher concentrations of CCC progressively increasing the response to GA_3, both in short days and in plants exposed to 1 long day. This synergism between GA_3 and CCC was apparent for all times of application of CCC between 2 days before and 6 days after the application of GA_3, but was greatest when both compounds were applied on the long day.

Applications of 4-hydroxy-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidinecarboxylate (Amo) reduced the flowering response to GA_3 and to 1 long day, in proportion to the amount applied. However, the reduction was independent of the time or method of application, and was related to the degree of leaf injury, suggesting that Amo did not specifically inhibit long-day induction.

The growth retardant *N*-dimethylaminosuccinamic acid (B_9) was inhibitory to flowering, but only at high concentrations (above 0·1M) and when applied either about 2 days before the long day or towards the end of the long day. B_9 was more inhibitory when injected than when applied to the leaves, suggesting action at the shoot apex. Its inhibitory effect was overcome by applied GA_3, but not by auxin.

Injections of chlorflurenole or abscisic acid were inhibitory to induction, but reduced the response to GA_3 only slightly.

5-Fluorodeoxyuridine was only slightly inhibitory to floral induction when applied on the long day, but eliminated the flowering response to GA_3 if applied within several hours of the GA_3 application.

Several explanations for these results are considered. It is concluded that endogenous gibberellins play no direct role in floral induction in *L. temulentum*, but that compounds sharing early steps in the biosynthetic pathway to gibberellins may do so, and that their action can be enhanced by applied gibberellins.

I. Introduction

The role of gibberellins in the induction of flowering in long-day plants remains unclear. Applied gibberellic acid (GA_3) can cause flowering in short days of a large number of long-day plants (Lang 1965, table 28). However, GA_3 also fails to induce

* Part XII, *Aust. J. biol. Sci.*, 1968, **21**, 1083–94.

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flowering in many long-day plants. In some cases the amount applied may have been too small, or the method of application not satisfactory. In others, the most effective gibberellin may not have been applied (cf. Michniewicz and Lang 1962). In some cases gibberellin application has suppressed flowering in long-day plants, as in *Fuchsia* (Sachs, Kofranek, and Shyr 1967), *Proserpinaca* (Davis 1967), and *Lemna gibba* (Cleland 1967).

To clarify the role of gibberellins in the induction of long-day plants, Baldev and Lang (1965) used growth retardants which interfere with gibberellin synthesis. These compounds reduced the flowering of *Samolus* following exposure to long days, and the higher their concentration the more long days or the more applied GA_3 was required to overcome the inhibition. These results suggested that applied gibberellins could substitute for long days, and that the critical effect of long days was to increase the level of endogenous gibberellins, while that of the growth retardants was to reduce it. A higher content of gibberellins following exposure to long days has been found in several plants (Chailahjan and Lozhnikova 1960, 1964, 1966; Okazawa 1960; Radley 1963). Radley (1963) found that, although exposure to only 1 long day caused an immediate rise in gibberellin level, this was transient, the level falling after further long days.

Lolium temulentum L. is a long-day plant which can be induced to flower by exposure to only 1 long day, or by the injection of only 3 μg of GA_3 per plant (Evans 1964*a*). GA_3 was the most effective gibberellin used. Its application also increased the flowering response of plants exposed to 1 long day, this effect being highly dependent on the time of application. The growth retardants, 4-hydroxy-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidinecarboxylate (Amo), and 2-chloroethyltrimethylammonium chloride (CCC) appeared to have no effect on induction, but they were applied only by injection, at moderate concentrations, and at the time of long-day induction. In the present experiments CCC, Amo, and another growth retardant, *N*-dimethylaminosuccinamic acid (B_9), were used at much higher concentrations and over a wider range of times, to analyse further the role of gibberellins in the induction of flowering in *L. temulentum*. Other compounds, such as chlorflurenole (2-chloro-9-hydroxyfluorene-9-carboxylate), abscisic acid, and 5-fluorodeoxyuridine (FDU), which may counteract the effect of applied gibberellins, were also used.

Amo retards growth by blocking the synthesis of gibberellins (Baldev, Lang, and Agatep 1965), probably by inhibiting the formation of kaurene from mevalonate (Dennis, Upper, and West 1965). CCC also blocks the synthesis of gibberellins, but probably acts at a much later step than Amo (Dennis, Upper, and West 1965; Harada and Lang 1965). The mode of action of B_9 is less clear. It does not prevent gibberellin action (Paleg *et al.* 1965). According to Ninnemann *et al.* (1964) B_9 did not reduce the level of gibberellins in *Fusarium*, but in *Echinocystis* a high concentration of B_9 reduced the incorporation of labelled mevalonic acid into kaurene by 41% (Dennis, Upper, and West 1965).

Chlorflurenole was found by Ziegler, Kohler, and Streitz (1966) not to affect gibberellin synthesis in *Fusarium*, but interacted with CCC and applied gibberellins in a way suggesting that it could be a competitive inhibitor of gibberellin action. However, subsequent work by Mann *et al.* (1966) and Tognoni, de Hertogh, and

Wittwer (1967) suggests that morphactins, like chlorflurenole, and gibberellins act independently. Abscisic acid, which can inhibit flowering in *L. temulentum* (Evans 1966; El-Antably, Wareing, and Hillman 1967), is known to interact with gibberellins in the control of bud dormancy (Eagles and Wareing 1964), α-amylase production (Chrispeels and Varner 1966, 1967), and germination and growth of the radicle in lettuce (Aspinall, Paleg, and Addicott 1967). FDU, which inhibits DNA synthesis, can also inhibit the response by hypocotyls and epicotyls to applied GA_3 (Nitsan and Lang 1965; Groves, Nitsan, and Lang 1966), the response being restored by the application of thymidine. Since FDU is only slightly inhibitory to flowering in *L. temulentum* when applied on the long day (Evans 1964*b*), it was also used here to examine the role of gibberellins in long-day induction.

II. Materials and Methods

Plants of the Ceres strain of *L. temulentum* were grown singly in pots of perlite at 25/20°C (day/night temperatures) under 8-hr days of natural light for about 5 weeks. All lower leaves and tillers were then removed, and the plants were exposed to 1 long day (day I) by extension of the 8-hr period of high-intensity light with incandescent light of 50 f.c. intensity for 16 hr. They were then returned to short days for a further 3 weeks before dissection of the apices of the primary shoots. Both the stage of differentiation reached, and the length of the apex above the base of the uppermost pair of overlapping leaf primordia, were recorded for the 10–14 plants in each treatment.

For all compounds, only single applications of freshly made solutions were used. The gibberellins, abscisic acid, chlorflurenole, B_9, and FDU were applied by injecting 0·1 ml of solution close to the shoot apex of each plant. All were in aqueous solution except chlorflurenole, which was in 10% ethyl alcohol solution, for which an appropriate control treatment was added.

Amo and CCC were applied in aqueous solution to the root medium. 10 ml were added to each pot, and the plants were not watered until 12 hr later, when they were leached with an excess of nutrient solution to remove residual retardant.

3-Indolylacetic acid (IAA), and in some experiments Amo and B_9, were applied by spraying the leaves with aqueous solutions, containing 0·1% Tween 20, at the rate of 1 ml/plant.

The gibberellins were supplied by I.C.I., and the equal mixture of GA_4+GA_7 by courtesy of Dr. J. MacMillan. CCC (98% pure) was a gift of American Cyanamid Co., B_9 of Naugatuck Chemical Co., chlorflurenole (the methyl ester IT3456) of Bayer Leverkusen, and FDU of Hoffmann-La Roche. Synthetic (±)-abscisic acid was kindly supplied by Dr. J. W. Cornforth.

III. Results

(*a*) *Effects of Applied Gibberellins*

The effect of the concentration of solutions of GA_3 injected at 4 p.m. on day I on both stem length and flowering response in plants kept in short days or exposed to 1 long day was examined in two experiments. Figure 1 presents the results with plants grown in high summer light intensities.

All the plants exposed to a long day initiated inflorescences, as did all of those kept in short days but treated once with GA_3, even at the lowest concentration, 1×10^{-4}M. The short-day controls, on the other hand, remained vegetative.

With plants exposed to a long day, both stem length and shoot apex length and stage of floral differentiation increased progressively with increase in GA_3 concentration. In plants treated with 12×10^{-4}M GA_3, shoot apex length was significantly ($P < 0{\cdot}01$) greater than in those treated with 4×10^{-4}M GA_3. With plants kept in short days, on the other hand, both stem length and apex length and stage approached

their maximum values with only 4×10^{-4}M GA_3, and neither stem nor apex length was significantly greater with 12×10^{-4}M GA_3.

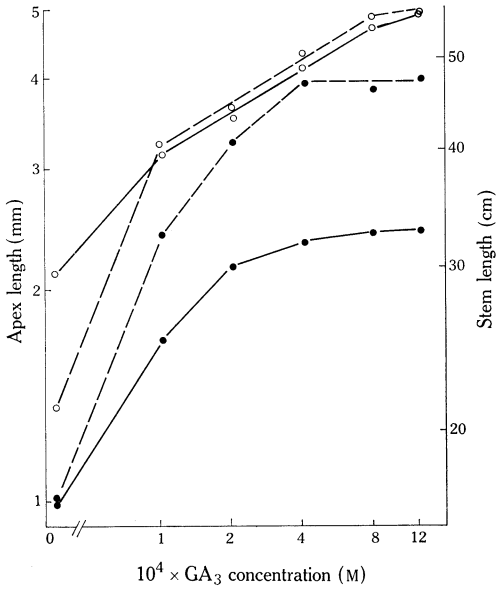

Fig. 1.—Effect of concentration of injected GA_3 on stem growth (– – –) and shoot apex length (——) in plants held in short days (●) or exposed to 1 long day (○).

In the experiment with plants grown in the lower intensity light of winter, this difference in response between plants exposed to a long day and those in short days throughout was even more marked (Table 1). For plants given a long day both stem growth and shoot apex development increased progressively with increase in concentration of GA_3 up to the highest level used, whereas there was a clear optimum at 4×10^{-4}M GA_3 for plants kept in short days, both stem and shoot apex length being lower at higher concentrations of GA_3.

TABLE 1

EFFECT OF CONCENTRATION OF INJECTED GIBBERELLIN SOLUTIONS ON STEM LENGTH (cm) AND SHOOT APEX LENGTH (mm) IN PLANTS KEPT IN SHORT DAYS OR EXPOSED TO 1 LONG DAY

Gibberellin	Day Length	Response	$10^4\times$ Total Gibberellin Concentration (M)			
			0	2	4	8
GA_3	Short days	Apex	0·76	1·28	1·98	1·69
		Stem	17	40	53	50
GA_3	1 long day	Apex	2·17	2·79	3·81	4·66
		Stem	21	37	50	56
GA_4+GA_7	Short days	Apex	0·76	1·07	1·39	1·32
		Stem	17	30	36	30
GA_4+GA_7	1 long day	Apex	2·17	2·25	2·67	3·32
		Stem	21	31	36	44

In this experiment, but not in the summer one, several abnormalities in inflorescence differentiation were evident in plants held in short days and treated

with either above or below optimal concentrations of GA_3. Also, at low concentrations of GA_3 (up to 2×10^{-4}M) not all plants initiated inflorescences. Table 1 presents the results with intermediate concentrations of GA_3 and of the equal mixture of GA_4+GA_7 in this experiment. As with GA_3 no optimum concentration of GA_4+GA_7 was evident for plants exposed to a long day, but for those in short days 4×10^{-4}M was optimal for both stem and apex length. In earlier experiments (Evans 1964*a*) GA_4 was found to have no effect on the flowering response of *L. temulentum* in either short or long days. Even if the effect of the equal mixture of GA_4+GA_7 was due wholly to GA_7, it is apparently less effective than GA_3 in this species. Nor is there any evidence of the pronounced synergism between GA_4 and GA_7 suggested by Ikuma and Thimann (1963). Treatments with mixtures of $GA_3+GA_4+GA_7$ gave responses slightly less than the additive effects of the separate components, with no evidence of synergism between them.

(*b*) *Experiments with CCC*

(i) *Concentration of CCC*

The effect of concentration of solutions of CCC applied to the root medium either 1 or 2 days before the long day has been examined in five experiments. The results for all five are in close agreement, and show the complete absence of any effect on flowering. Growth of the plants was little affected by concentrations of 0·01M CCC or less, but was increasingly inhibited at higher concentrations, in agreement with the results of Stoddart (1965). The lengths of stems and of both sheaths and blades of the upper leaves were reduced by up to 50% with 0·3M CCC. At this concentration the shoot apex on nearly all plants was killed and the plants were severely injured, while 1M solutions rapidly killed the plants. 0·1M was the highest concentration which could be used without killing the shoot apices, and this concentration greatly reduced stem and leaf length and induced a variety of abnormalities: the stems became very brittle, anthocyanins accumulated in the lower part of the leaf sheaths, white patches developed on the leaf blades which were otherwise a very dark green colour, and an abundance of smaller tillers developed, even at positions high up the stem.

Despite these very pronounced effects on growth, no effect of CCC on the induction of flowering and inflorescence development was seen in any experiment, even those in which long-day induction was minimal.

(ii) *Time of Application*

CCC solutions of various high concentrations have been applied to plants exposed to 1 long day, at times ranging from 3 days before to 6 days after the long day, in six experiments. The results from three experiments are given in Figure 2. At no time, in any experiment, has CCC had a significant inhibitory effect on apex length or stage of differentiation. This was true even in the experiments carried out in winter, when the flowering response to exposure to a long day was small. Baldev and Lang (1965) found CCC to be more inhibitory the more marginal was induction.

(iii) *Interactions between CCC and GA_3*

In two experiments, a factorial combination of four concentrations of CCC applied at 4 p.m. on the day before the long day (—I) with four concentrations of

GA_3 injected at 4 p.m. on the long day (I) was examined. Plants held continuously in short days were given the same combination of treatments. The results from the two experiments were similar, and those from one are presented in Figure 3.

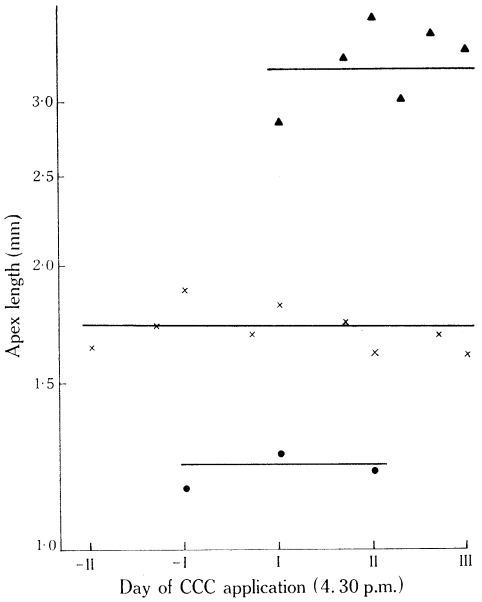

Fig. 2.—Effect of time of application of CCC on the flowering response of plants exposed to 1 long day, in three experiments. The solid lines represent the response of the controls (no CCC) exposed to a long day on day I.
× 0·2M CCC (June);
● 0·1M CCC (August);
▲ 0·03M CCC (February).

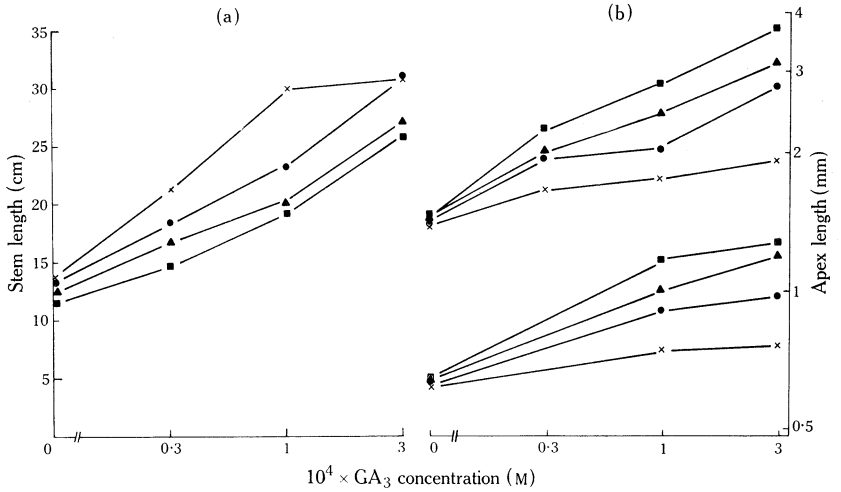

Fig. 3.—Effect of concentration of CCC applied the day before the long day and of GA_3 injected on the long day on stem length (*a*) and shoot apex length (*b*). Shoot apex length of plants kept in short days is shown in the lower set of curves in (*b*). × No CCC. ● 0·01M CCC. ▲ 0·03M CCC. ■ 0·1M CCC.

The effects on stem length [Fig. 3(*a*)] were as expected: the higher the concentration of GA_3 the greater the length, with the opposite relation for CCC. GA_3 at the highest concentration could overcome the dwarfing effect of 0·01M CCC, but

some effect of the higher CCC concentrations remained. This pattern of results is like that of Baldev and Lang (1965), and is readily explainable on the basis that CCC reduces stem length by reducing the level of endogenous gibberellins, and that these can be replaced by exogenous GA_3.

The effects on flowering response [Fig. 3(*b*)], however, were quite different, GA_3 and CCC acting synergistically rather than antagonistically. Shoot apex length increased with increasing concentration of GA_3, and also with increasing CCC concentration when GA_3 was also applied, irrespective of whether the plants were in short days or given 1 long day. The synergistic effect was very great, and was apparent not only in the length of the apex, but also in the stage of floral differentiation reached. For example, while the short-day controls were all vegetative, as were the plants treated with CCC alone, one-third of those in short days and treated with 3×10^{-4}M GA_3 reached double ridges, while those treated with $0\cdot1$M CCC$+3\times10^{-4}$M GA_3 all passed the double ridges stage, some even differentiating lemma primordia. The long-day controls all differentiated lemma primordia, many of those treated with 3×10^{-4}M GA_3 differentiated floret primordia, while many of those treated with $0\cdot1$M CCC$+3\times10^{-4}$M GA_3 had differentiated anther primordia at the time of dissection.

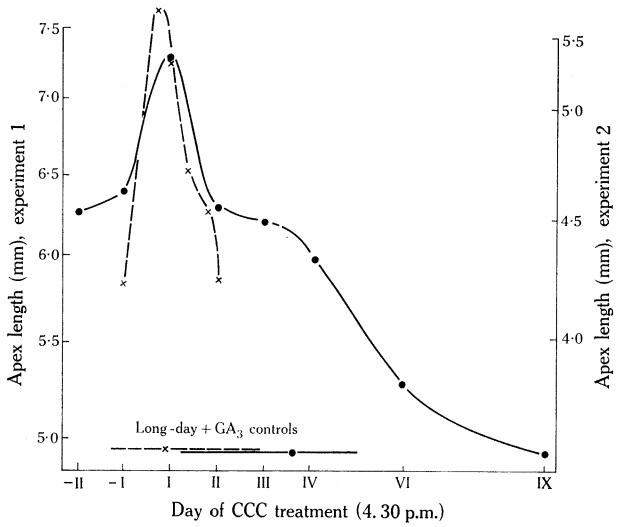

Fig. 4.—Effect of time of application of CCC (3×10^{-2}M) on inflorescence length in plants injected with GA_3 (3×10^{-4}M) at 4 p.m. on the long day (I) in two experiments.
× Experiment 1.
● Experiment 2.

The relative times of application of GA_3 and CCC were varied in two experiments, the results of which are given in Figure 4. In both, the synergism between GA_3 and CCC was greatest when the CCC, as well as the GA_3, was applied on the long day. However, the synergism was still marked when GA_3 was injected on the long day, and the CCC applied 3 days later, by which time the plants were beginning to differentiate spikelet primordia. In other treatments in which the GA_3 was applied on day II and CCC on day I marked synergism was also evident.

(*c*) *Treatments with Amo*

Amo solutions ranging in concentration from 3×10^{-3} to $0\cdot1$M were applied to the leaves at 4 p.m. on day −I. The flowering response to 1 long day was progressively

smaller the higher the concentration of Amo applied. However, this reduction in flowering response was accompanied by leaf injury, which was severe and evident even before exposure to the long day at concentrations of 0·03M and higher.

The reduction by Amo in the flowering response to 1 long day was independent of the time of application between day —II and day III. It was also independent of the mode of application, via leaves or roots, for a given amount of Amo per plant.

When Amo was applied to plants exposed to 1 long day and injected with GA_3 at 4 p.m. on day I, spray applications between day I and day III all reduced the flowering response to a similar small extent, while root applications between day —I and day III had little effect.

The fact that the inhibitory effects of Amo on the flowering response were independent of the time of application, and were related to the degree of leaf injury, suggest that Amo did not specifically inhibit long-day induction.

Fig. 5

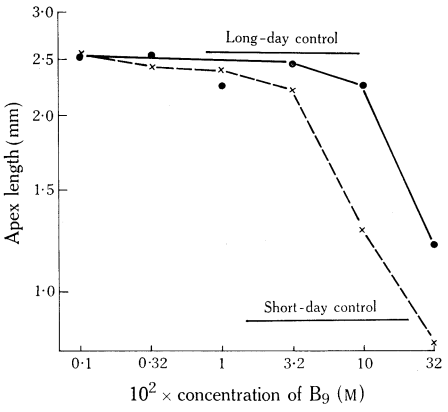

Fig. 6

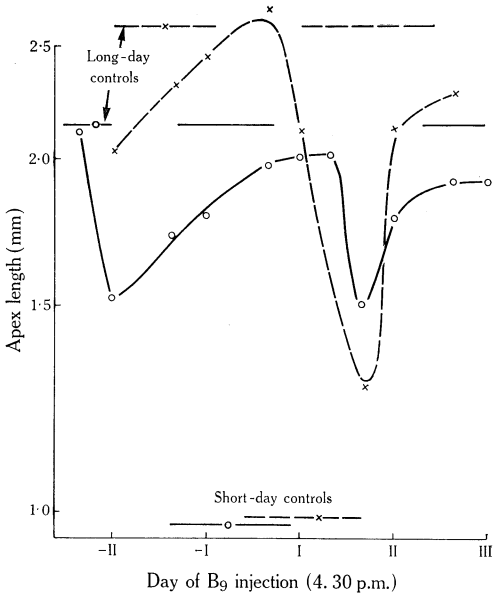

Fig. 5.—Effect of concentration of B_9 solution on shoot apex length in plants exposed to a long day for applications at 9 a.m. on day II. ● 1 ml/plant sprayed on leaves. × 0·1 ml/plant injected near apex.

Fig. 6.—Effect of time of injection with B_9 (0·1M) on inflorescence length in plants exposed to 1 long day (I) in two experiments. ● Experiment 1. × Experiment 2.

(*d*) *B_9 Treatments*

The effect of concentration of B_9 solutions applied at 9 a.m. on day II on the flowering response of plants exposed to 1 long day is shown in Figure 5. With spray applications to the leaves, no injury was apparent at concentrations of $3{\cdot}2\times10^{-2}$M or less, but was marked at the higher concentrations, particularly in the loss of apical

dominance. Similarly, B_9 was inhibitory to flowering only at the two highest concentrations with leaf sprays. It was more inhibitory to flowering when injected, despite the smaller application per plant. The same was true for B_9 applications to *Pharbitis* (Zeevaart 1966), and suggests action at the shoot apex rather than in the leaf. However, more than 10 times as much B_9 was required to reduce the flowering response by 50% in *L. temulentum* as in *Pharbitis*.

The effect of time of application of B_9 to plants exposed to a long day was examined in four experiments. The results from two are given in Figure 6. In all the experiments B_9 was particularly inhibitory at two times of application, whether applied as spray or by injection. The first time was 2 days before the long day (day —II). Applications on the day before the long day were consistently less inhibitory, and those early on the long day least inhibitory. The inhibitory effect of B_9 then increased rapidly, usually being greatest for injections made at 9 a.m. on day II. This second peak of inhibition varied somewhat between experiments, and in one of them occurred at 11 p.m. on day I. In this case, however, injections early on day II were still highly inhibitory.

TABLE 2

INTERACTIONS BETWEEN B_9 AND GA_3 APPLICATIONS ON SHOOT APEX LENGTH (mm) IN PLANTS EXPOSED TO 1 LONG DAY

6×10^{-2}M B_9 and 3×10^{-4}M GA_3 injected

B_9 Treatment	GA_3 Treatment		
	None	4 p.m. Day I	4 p.m. Day II
None	1·60	2·50	2·55
4 p.m. day —II	1·24	2·08	—
9 a.m. day II	1·04	1·70	1·80

The inhibitory effect on flower induction of B_9 injections 2 days before, or the day after, the long day could be overcome by applications of GA_3 but not by auxin, as Zeevaart (1966) found with *Pharbitis*. 1×10^{-3}M IAA was sprayed on the leaves at times when auxin is not inhibitory to induction in *L. temulentum* (Evans 1964*a*), namely 9 a.m. on day I for B_9 injections at 4 p.m. on day —II, and noon on day II for B_9 injections at 9 a.m. on day II. In both cases the inhibitory effect of B_9 was accentuated rather than diminished by IAA, especially with B_9 applied on day —II. The results for the B_9 and GA_3 treatments in the same experiment are given in Table 2. The inhibitory effect of B_9 applied either before or after the long day was overcome by subsequent GA_3 applications, shoot apex length being intermediate between that on plants treated with B_9 alone and that with GA_3 alone. Such a result could be due either to independent action of B_9 and GA_3, or to GA_3 replacing the substance inhibited by B_9.

(*e*) *Chlorflurenole*

The experiment with chlorflurenole was carried out in winter, when the response to induction by 1 long day is minimal. Despite this, although injections of chlor-

flurenole ($1 \cdot 5 \times 10^{-5}$ to $4 \cdot 5 \times 10^{-4}$M) between 2 p.m. on day I and 9 a.m. on day II reduced shoot apex length in all cases, they did not prevent floral initiation. All plants initiated spikelets, the reduced apex length being due to abnormal differentiation at the summit of the shoot apex. The uppermost leaf primordium formed a circular collar which overtopped the summit, presumably because of inhibition of summit activity. When GA_3 was applied as well as chlorflurenole, shoot apex length was restored beyond the level of the long-day controls, but the summit abnormality was still present in most plants. This suggests that the action of chlorflurenole is at least partly independent of GA_3 action, as Mann *et al.* (1966) and Tognoni, de Hertogh, and Wittwer (1967) have also concluded. These treatments are of interest, however, in that despite the apparent stasis of the summit of the apex due to chlorflurenole, the spikelets induced at pre-existing sites on the apex could reach the stage of floret differentiation when GA_3 was added. Thus, induction and differentiation at these sites does not always require the presence of an active summit.

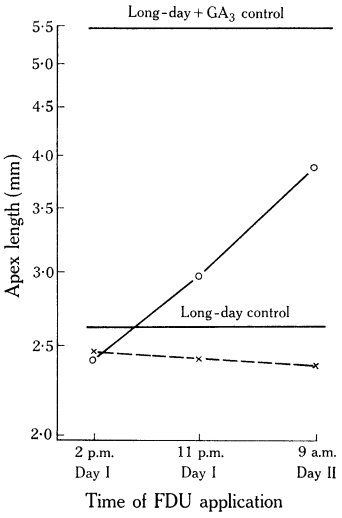

Fig. 7.—Effect of time of injection with FDU ($2 \cdot 5 \times 10^{-4}$M) on inflorescence length of plants exposed to a long day (I).
× Injected with FDU alone.
○ Also injected with GA_3 (4×10^{-4}M) at 4 p.m. on day I.

(*f*) *5-Fluorodeoxyuridine Treatments*

Three experiments explored the interaction between FDU and GA_3 on the flowering response to a long day. FDU had previously been shown to have only a slight inhibitory effect on long-day induction in *L. temulentum* when applied on day I, when GA_3 was having its greatest effect (Evans 1964*a*, 1964*b*). In the present experiments also, FDU injections on day I and early on day II reduced apex length only slightly (Fig. 7), and the stage of floral differentiation not at all. Nevertheless, FDU injected 2 hr before GA_3 completely eliminated the flowering response to GA_3, in all three experiments. The results in Figure 7 show that the promotive effect of GA_3 rapidly escapes from inhibition by FDU, and this was confirmed in another experiment. This implies that the action of GA_3 on the flowering processes is quickly consummated.

FDU injected 2 hr before 4×10^{-4}M GA_3 (injected at 4 p.m.) also eliminated the flowering response to GA_3 by plants held continuously in short days, as shown in the following tabulation:

	GA_3	$10^4\times$FDU Concentration		
		0	1	2·5
Percentage initiation	−	0	0	0
Apex length (mm)	−	0·83	0·71	0·66
Percentage initiation	+	100	45	0
Apex length (mm)	+	2·29	1·59	0·84

(*g*) *Abscisic Acid*

Synthetic (±)-abscisic acid was applied at various times and concentrations to plants exposed to a long day, by injection near the shoot apex. Detailed results will not be presented, since the time course of inhibition was almost identical to that found previously with the natural product (Evans 1966). Abscisic acid was most inhibitory when applied at 9 a.m. on day II, and was not inhibitory when applied early on the long day, or 2 days after the long day. Abscisic acid (7×10^{-5} to 2×10^{-4}M)$\times GA_3$ (10^{-4}M) interactions were examined in two experiments; in both, abscisic acid reduced only slightly the flowering response to GA_3 applied at 4 p.m. on the long day.

IV. Discussion

The results presented previously (Evans 1964*a*) and above show that single applications of GA_3 can induce flowering in *L. temulentum* plants kept in short days. The flowering response increased with GA_3 concentration up to 4×10^{-4}M, at which all treated plants initiated inflorescences whose stage of differentiation at dissection ranged from glume to floret primordia, depending on light-intensity conditions. GA_3-induced floral development differed from that induced by exposure to a long day mainly in the gradient of differentiation down the shoot apex. With long-day induction the most advanced spikelets were the terminal one and those one-third to half way down the apex; with GA_3 induction the lowest spikelets were the most advanced, and the terminal spikelet was sometimes absent, the summit appearing to revert to the vegetative condition. There was no evidence that other gibberellins, such as GA_7, were more effective than GA_3, or that there was synergism between GA_3, GA_4, and GA_7.

In view of these results, and of the conclusion by Baldev and Lang (1965) that long-day induction in *Samolus* acts by increasing the level of endogenous gibberellins, is long-day induction in *Lolium* mediated by the gibberellins? Several lines of evidence suggest it is not.

(1) If the operative effect of the long day is to raise the level of endogenous gibberellins, we would expect the promotive effect of applied gibberellins to be greater on plants in short days than on those exposed to a long day, or at least to increase up to higher concentrations of applied GA_3, whereas the opposite is the case (Fig. 1 and Table 1).

(2) Applications of CCC, which inhibits gibberellin synthesis, had no inhibitory effect on the flowering response to a long day at any time of application, even at the highest concentration which did not kill the shoot apex. Such applications were very inhibitory to vegetative growth, and were shown by Stoddart (1965) to have profound effects on carbohydrate metabolism in plants of *L. temulentum*. The small reductions in flowering response due to application of Amo, which also inhibits gibberellin synthesis, were probably due to injury rather than to a specific inhibition of long-day induction.

(3) FDU applied at 2 p.m. on day I had only a very slight inhibitory effect on induction by exposure to a long day, yet eliminated the response to applied GA_3. Thus, if the action of endogenous gibberellins requires DNA synthesis, as that of GA_3 appears to do, then gibberellins are unlikely to play any direct role in the long-day induction of flowering in *L. temulentum*.

In the terminology of Baldev and Lang (1965), the inductive effect of GA_3 in *Lolium* is more likely pharmacological than physiological, whereas in *Samolus* the opposite was their conclusion. Also opposite in these two long-day plants is the interaction between CCC and GA_3 in their effect on the flowering response: in *Samolus* they were antagonistic, in *Lolium* synergistic. A comparable synergism between CCC and GA_3 has been found for internode elongation in the strawberry (Guttridge 1966) and stem elongation in *Scrophularia* (Groves and Lang, unpublished data). Since the synergism in *Lolium* was so marked, and so different from the relation between CCC and GA_3 in *Samolus*, several possible explanations for it will be considered.

(1) Exogenous GA_3 may be more effective in increasing the flowering response than are endogenous gibberellins, which may compete with GA_3 at the site of gibberellin action. These being suppressed by CCC, GA_3 becomes more effective. CCC alone would have no effect if the endogenous gibberellins played no role in flower induction.

This explanation is rendered unlikely by the fact that, despite the evidence for rapid GA_3 action (Fig. 7), CCC is still synergistic when applied even 3 days after the GA_3 (Fig. 4).

(2) CCC particularly inhibits growth of stems and leaves, and may lead to reduced competition with the shoot apex for substrates, allowing the apex to respond more to the long-day stimulus and to applied GA_3. However, there was no evidence that severe reduction of growth by CCC led to greater apex development, whereas GA_3 greatly increased *both* stem growth and apical development.

(3) There may be a well-defined optimum gibberellin level, and while applied GA_3 may raise this beyond the optimum, CCC could restore it to the optimum, and have an apparently synergistic effect. An optimum was evident for applications of GA_3 to plants in short days under low light intensities (Table 1). The presence of such an optimum could explain why GA_3 inhibited flowering of the long-day plants *Fuchsia* (Sachs, Kofranek, and Shyr 1967), *Proserpinaca* (Davis 1967), and *Lemna gibba* (Cleland 1967) in long days. However, no such optimum GA_3 concentration was evident with *L. temulentum* plants exposed to a long day, and the $GA_3 \times CCC$

synergism occurred with GA_3 concentrations well below the highest used. It also occurred when the CCC was applied long after the GA_3 had probably acted.

(4) CCC may cause other effects besides a reduction in the level of endogenous gibberellins, and these may influence the inductive processes. CCC can reduce the levels of tryptophan and auxin in plant tissue (Norris 1966), and at least some of its effects can be counteracted by applied auxin but not by gibberellins (Khan and Tolbert 1966). $GA_3 \times$ CCC synergism can occur in the reduction of IAA-oxidase activity in hypocotyls (Knypl and Rennert 1967), when the two compounds are supplied simultaneously.

However, if the effect of CCC on flowering was independent of its effect on gibberellin level, it is difficult to see why CCC alone had no effect on flowering over a wide range of concentrations and times of application.

(5) The following more complex model can account for most of the findings reported in this paper. It is assumed that:

(i) Early steps in the synthesis of the gibberellins and of the floral stimulus in *Lolium* lie on a common pathway.

(ii) B_9 blocks one of these early steps.

(iii) CCC blocks a late step in gibberellin synthesis, and in doing so increases the flow of an early precursor into the floral-stimulus pathway. A comparable example is that of Amo, which blocks the synthesis of kaurene on the gibberellin pathway and thereby causes a large increase in the amount of mevalonic acid incorporated into *trans*-geranylgeraniol on an alternative pathway (Dennis, Upper, and West 1965).

(iv) The long-day stimulus in *Lolium* not only causes floral evocation, but also accelerates differentiation during floral development, as concluded elsewhere (Evans 1969).

(v) Gibberellins, while not playing a direct role in flower induction, can enhance the response to the floral stimulus, as has been suggested by Carr (1967). They could do this, for example, by increasing the rate or synchronization of cell division in the apex (cf. Sachs and Lang 1961), or by increasing apical dominance and the flow of materials into the apex (cf. Ruddat and Pharis 1966).

This model explains why B_9 is inhibitory to flower induction (ii) when CCC is not (iii), and why B_9 can be inhibitory even when applied after the long day (iv; ii and v). It explains why CCC and GA_3 have a synergistic effect on the flowering response (iii, v), even when the CCC is applied after GA_3 and long-day induction (iv). On this basis GA_3 could eliminate the inhibitory effect of B_9, not only by replacing the inhibited compound (a gibberellin), but also by enhancing the action of the reduced amount of floral stimulus (ii, v). The induction of flowering in short days by GA_3 would be by a similar mechanism, GA_3 enhancing the action of the normally subthreshold amount of floral stimulus present in short days. The absence of any net effect on the flowering response of CCC alone could be due to it increasing the

amount of floral stimulus (iii) on the one hand, but reducing the enhancement of its action by endogenous gibberellins (v) on the other.

V. Acknowledgments

I am grateful to Mrs. Katie Bretz for technical assistance with these experiments, to the individuals and companies who supplied the compounds used in them, and to my colleagues Drs. R. H. Groves, J. V. Jacobsen, and R. B. Knox for many perceptive comments.

VI. References

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