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Gibberellins in Relation to Flowering and Stem Elongation in the Long Day Plant *Silene armeria*[1]

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ABSTRACT

Two long days induced some flowering and 4 or more long days caused 100% flowering in *Silene armeria*. On long days microscopically detectable flower primordia were first seen after 6 days, which is at least 1 day before the start of stem elongation. Both gibberellin A_3 and A_7 caused flowering on short days, but the results were variable and flowering was never 100%. Three different gibberellins were detected in *Silene* extracts. The pattern of gibberellins extracted from plants on short and long days was qualitatively the same, but on long days gibberellin content was up to 100% higher than on short days. Only small amounts of diffusible gibberellins were obtained from *Silene* shoot tips (including very young leaves) on short days. However, on long days the diffusible gibberellins increased by as much as 10-fold after 4 to 6 long days but then declined somewhat after 10 long days. The gibberellins extracted from the shoot tips at the completion of the diffusion period also increased under long days, although the increase was not as large as for the diffusible gibberellins. An A_5-like gibberellin present in extracts was not detected in diffusates.

Treatment with AMO 1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride) completely inhibited stem elongation on long days but had no effect on flowering. In addition, treatment with AMO 1618 caused at least an 80% decrease in the level of extractable gibberellin, while the diffusible gibberellin was reduced below the limits of detection in the d5 corn bioassay. When endogenous gibberellin levels were suppressed by pretreatment with AMO 1618 on short days, gibberellin A_3 caused more stem elongation in plants moved to long days than in plants left on short days. Thus the sensitivity of *Silene* plants to gibberellin with respect to stem growth is affected by photoperiod. It is concluded that in *Silene* endogenous gibberellins are a controlling factor for stem elongation but apparently are not required for flower formation.

Treatment on short days with gibberellic acid leads to stem elongation and flower formation in many rosette long-day plants (19, 21). In addition, flowering can also be induced by gibberellin treatment under SD[3] in the long-short-day plant *Bryophyllum daigremontianum* (46) and under long days in the short day plant *Impatiens balsamina* (30). In each case flower induction, whether by photoperiodic treatment or GA treatment, is accompanied by considerable stem elongation. By contrast, in most SDP and nonrosette LDP where flowering is not associated with significant stem elongation, GA treatment does not lead to flower formation (9, 21) or may even be inhibitory to flowering (9, 13, 34).

In recent years additional evidence for the possible involvement of GA in the regulation of flowering has come from studies with the plant growth retardants CCC and AMO 1618, which are known to inhibit endogenous GA biosynthesis (3, 10, 31, 33). Flowering in the rosette LDP *Samolus parviflorus* (1), the nonrosette LDP *Lemma gibba* G3 (9), the SDP *Pharbitis nil* (44) and the LSDP *Bryophyllum* (43) could be inhibited under inductive conditions by the addition of CCC or AMO 1618, and this inhibition was reversed by simultaneous application of GA. However, in certain other plants the application of CCC or AMO 1618 apparently does not have any inhibitory effect on flowering (4, 36).

Numerous attempts have been made to examine the endogenous GAs obtained by extraction from plants grown under a variety of environmental conditions and to relate them to flowering (7, 11, 12, 20, 25, 48). In general, it has been found that the level of extractable GA is higher on LD than on SD. Harada (11, 12) was able to detect increases in GA-like substances prior to the start of flower formation in several different plants. In the LSDP *Bryophyllum* the transfer of plants from LD to SD resulted in an increase in the GA content prior to the formation of flower primordia (48). However, in most other studies plants kept under inductive photoperiods were not extracted until after the appearance of buds and flowers (7, 8, 23, 25). In addition, the increase in GA with LD treatment has been obtained for both LDP and SDP (7, 8). Thus, the significance of these changes in endogenous GAs for the regulation of flower formation remains doubtful.

The rosette LDP *Silene armeria* could be induced to flower on SD by GA_7, but other GAs were ineffective (26), or effective only at very high doses (40). Preliminary studies indicated that endogenous GAs could be obtained from *Silene* either by extraction or by diffusion from shoot tips by the agar diffusion technique (16, 17). Extraction yields higher levels of GA activity but provides information only on the GA content at any given time. In order to

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[3] Abbreviations: SD: short days; LD: long days; LDP: long day plants; LSDP: long-short day plants; SDP: short day plants; CCC: (2-chloroethyl)trimethylammonium chloride; AMO 1618: 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride.

get some estimate of GA production and metabolism by a given plant organ, it is necessary to examine also the GAs obtained by diffusion. Therefore, a study was undertaken to examine both the extractable and diffusible GAs obtained from *Silene* plants growing under various environmental conditions in an effort to correlate levels of endogenous GAs with stem elongation and flower formation in this particular rosette LDP.

MATERIALS AND METHODS

Plant Material. An inbred strain of *S. armeria* L. (41) was the same as used in earlier work (19, 24, 26, 39, 40) with this species. Seeds were germinated in soil and when plants were several weeks old they were transplanted into a gravel-vermiculite (1:2) mixture and watered daily with half-strength Hoagland nutrient solution. The plants were grown in a greenhouse maintained at a minimal temperature of 22 C on SD consisting of 8 hr of natural light and 16 hr of darkness in a dark room (temperature 15 C). They were used for experiments when 2 to 3 months old. Under these conditions the plants formed numerous axillary shoots. For extraction and diffusion experiments these shoots were left intact, but for all other experiments they were removed at the start of the experiment and at periodic intervals thereafter.

Experiments involving GA applications were carried out in the greenhouse. All other experiments were carried out in growth chambers maintained at 23 C with an 8-hr light period of mixed fluorescent and incandescent light (3000 ft-c) followed by 16 hr of darkness for SD, or a 16-hr day length extension with light from incandescent bulbs (40 ft-c) for LD. After transfer to the growth chambers the plants were given several SD prior to the start of an experiment.

Flowering was scored by the presence or absence of flowers or flower primordia on the main shoot axis. A plant was considered to be flowering if upon dissection the stem apex was at least at stage I of floral primordia development (37). For determination of stem height the distance from the base of the main stem axis (usually easily identified by a slight constriction of the stem) to the tip of the vegetative stem or base of the flowering inflorescence was measured.

GA and AMO 1618 Applications. The GAs used were GA_3 (97% pure, courtesy of Merck & Co.) and GA_7 (Nutritional Biochemical Corp.) which contained approximately 20% GA_4 as a contaminant. The GAs were dissolved in a 0.05% Tween 20 solution containing 5% ethanol. They were applied as 0.05-ml drops on alternate days to the shoot apex.

AMO 1618 was used at a concentration of 10^{-2} M, and 10 ml were applied to the soil on alternate days.

Extraction Procedures. Plants were harvested toward the end of the main 8-hr high intensity light period. Unless otherwise indicated, the entire plant above ground level was extracted. The plant material was frozen in liquid nitrogen, lyophilized, and extracted with cold methanol in a Waring Blendor. After filtration the residue was stirred with additional methanol overnight and filtered again. The combined filtrate was evaporated to dryness under reduced pressure. The residue was taken up in 0.5 M phosphate buffer (pH 8.2) and partitioned several times with petroleum ether and then four to six times with equal volumes of ethyl acetate until the organic phase was colorless. The buffer was acidified to pH 2.5, centrifuged at low speed to remove precipitate, and then extracted three times with ethyl acetate. The combined ethyl acetate phase was dried over Na_2SO_4 and evaporated to near dryness.

Diffusion Technique. The main shoot and all axillary shoots at least 1 cm long (including leaf length) were used for diffusion. All mature leaves were removed, leaving only two to three easily visible leaf pairs. The shoots were cut about 1 cm below the apex (less if shoot length less than 1 cm) and placed in 1.5% agar containing 0.01 M KH_2PO_4, pH 5.5. Each treatment consisted of several hundred shoot tips from 20 to 30 plants that were placed in agar in a single plastic refrigerator box (9 x 19 x 8 cm) that was kept covered during the diffusion period. The shoot tips were allowed to diffuse for 24 hr in darkness at 27 ± 1 C. At the end of the diffusion period the shoot tips were removed from the agar, frozen in liquid nitrogen, and extracted as described above.

The agar was placed in a flask and frozen. Methanol was added to the frozen agar, and the flask was left at room temperature overnight. The next day the liquid was filtered and the methanol was evaporated off under reduced pressure. The remaining aqueous phase was acidified to pH 2.5 and partitioned three times with ethyl acetate. The combined ethyl acetate phase was dried over Na_2SO_4 and evaporated to near dryness.

Chromatography. Preparative thin layer chromatography on Silica Gel H was carried out according to Sembdner *et al.* (35). The acidic fraction was applied as a 0.5- to 1.0-cm band to a 20- × 20-cm glass plate prepared with a 0.4-mm layer of Silica Gel H. Solvent system 1 (35) consisting of chloroform-ethyl acetate-acetic acid (60:40:5, v/v) was used and was permitted to run a distance of 15 cm. The chromatogram was divided into 10 equal zones, and the silica gel was scraped into centrifuge tubes and eluted three times with water-saturated ethyl acetate. The eluate was evaporated to dryness and used for the bioassay.

Bioassay. The dwarf corn bioassay (d5) was used as described by Kende and Lang (18), utilizing homozygous d5 seed (45).

Cochromatography of *Silene* GAs with Authentic GAs. After preparative thin layer chromatography of extracts in solvent system 1 two zones with GA activity in the d5 corn bioassay were detected. These were indicated as Silene I (R_F 0.1–0.3) and Silene II (R_F 0.4–0.6). Each of these fractions was rechromatographed in a different solvent on 250-μ thick Silica Gel H plates which had been activated at 120 C. Fractions were applied as a narrow band 15 cm long. Authentic GAs were spotted in the middle of this band. To improve resolution, the plates were predeveloped with acetone-acetic acid (100:1, v/v) until the zone of application was in a narrow line about 0.5 cm from the origin (38). Silene I was applied to 20- × 40-cm plates and developed to 30 cm in solvent system 2 (38), which consisted of diisopropyl-ether-acetone-acetic acid (90:30:1, v/v).

Silene II was rechromatographed in solvent system 3 (14). To prepare this solvent, carbon tetrachloride-acetic acid-water (8:3:5, v/v) were shaken in a separatory funnel and separated into two phases. The plates were equilibrated with the upper phase overnight and then developed with a mixture of the lower phase (5 parts) and ethyl acetate (1 part). The plates were developed to 15 cm.

One-centimeter zones were scraped off and tested for biological activity on d5 corn. A central zone across the plates with the reference GAs was left intact and sprayed with 5% (v/v) H_2SO_4 in ethanol, heated at 120 C for 10 min, and fluorescent GA spots were localized in ultraviolet light.

When GAs obtained by diffusion and extraction from shoot tips were compared with authentic GAs, the acidic fraction from diffusates was sufficiently clean to run immediately in solvent system 3. However, for extracts of shoot tips the acidic fraction was first chromatographed in solvent system 1. Then the 1.5- to 4-cm zone was rechromatographed in benzene-*n*-butanol-acetic acid (70:25:5, v/v). The 4.5- to -9 cm zone from this plate was eluted and combined with the eluate of the 4- to 10-cm zone from the first plate. The combined eluates were then cochromatographed in solvent system 3 with authentic GAs.

RESULTS

Photoperiodic Requirements for Flowering. Initial studies were undertaken to determine the minimal number of LD needed to induce flowering. From the results of Table I it is apparent that 4 or more LD gave 100% flowering, while as few as 2 LD were

Table I. *Number of Long Days Required for Flower Induction*
Plants examined 8 weeks after start of long day treatment.

Experiment	No. of Long Days	Stem Height	Flowering[1]
		cm	
1	0	4.1 ± 0.3	0/9
	2	7.9 ± 2.1	1/9
	3	16.6 ± 2.8	6/9
	4	19.3 ± 2.1	9/9
	5	18.2 ± 1.6	8/9
	6	26.2 ± 2.0	9/9
2	2	9.6 ± 4.8	3/6
	3	17.5 ± 2.4	5/6
	4	19.9 ± 2.4	6/6
	5	27.1 ± 2.4	6/6
	6	31.5 ± 3.1	6/6

[1] No. of plants flowering/No. of plants examined.

Table II. *Influence of Increasing Number of Long Days on Stem Elongation and Flowering*
Plants examined at end of long day treatment.

No. of Long Days	Stem Height	Flowering[1]
	cm	
0	...	0/6
5	2.6 ± 0.1	0/6
6	2.4 ± 0.2	4/6
7	3.1 ± 0.2	6/6
8	3.7 ± 0.2	6/6

[1] No. of plants flowering/No. of plants examined.

sufficient to induce some flowering. The minimal number of LD needed for maximal stem elongation was not determined, but it is clearly 6 or more LD. With only 2 LD those plants which remained vegetative showed slightly more stem elongation than did the SD controls, but substantial stem elongation occurred only in those plants that were induced to flower.

When plants were transferred to LD, the first appearance of microscopically detectable flower primordia was after 6 days (Table II). Thus the onset of floral initiation precedes the beginning of measurable stem elongation by 1 or more days (see also Table VIII).

Influence of Applied GAs. Contrary to published reports (26, 40) GA_3 and GA_7 were equally effective for induction of flowering (Table III). However, the results were quite variable, with little or no flowering in some experiments. Furthermore, within a given experiment there was considerable variation both in the flowering response, which was never 100%, and in the final stem height as indicated by the standard errors.

When GA_3 was given on alternate days over a period of 3 to 4 weeks, the minimal dose required to obtain some flowering was 1 to 10 μg (Table III, experiment 3). However, when as much as 60 μg of GA_7 were given over a shorter time period, there was no flowering (Table III, experiment 2). Thus, to obtain flowering, it is necessary to use fairly high dosages of GA given over a period of several weeks. When plants were examined as soon as the GA treatments were completed (Table III, experiment 2), it was clear that the GA first stimulated substantial stem elongation and only later were flower primordia formed.

GA Extractions. Several experiments were carried out to investigate the influence of photoperiod on the levels of extractable GA (Table IV). On SD significant GA activity was obtained in two zones of the thin layer chromatograms indicated as Silene I (R_F 0.1–0.3) and Silene II (R_F 0.4–0.6) (Fig. 1). After exposing plants to LD, the same two zones of activity were present, but there was an increase in the over-all level of extractable GA in most experiments. The total increase in GA activity varied from no increase (experiment 3) to a 3-fold increase (experiment 2), but in most experiments the increase was somewhat less than 2-fold. Examination after one LD did not yield a dramatic increase in GA activity as has been reported for spinach (32). Rather, considering all experimental data there seemed to be a gradual increase which reached a peak after exposure to several LD.

When different parts of the plant were extracted separately, the GA activity proved to be much higher in the shoot tips (including very young leaves) as compared to leaves and stem tissue (Table V). However, the increase obtained upon transfer to LD was similar in both cases.

GA Diffusion. The influence of photoperiod on the amount of diffusible GA obtained from shoot tips is shown in Table VI. On SD the level of diffusible GA was quite low, but after 4 to 6 LD there was a several-fold or greater increase. However, after 8 to 10 LD the amount of diffusible GA had decreased again. The level of GA extracted from shoot tips after the diffusion period also showed a significant increase after 4 or more LD, but this increase was usually less than 3-fold and thus not as marked as in the case of diffusible GA.

The extractable GAs from *Silene* showed two zones of activity in solvent system 1 (Fig. 1). In this same solvent system the diffusible GAs also showed two zones of activity (Fig. 2), but the R_F values were slightly lower (0.1–0.2 and 0.3–0.4 as compared to 0.2–0.3 and 0.4–0.5, respectively, for the GAs obtained by extraction). It would seem that these small differences in R_F values were due to contaminating organic acids which are always present in extracts, since rechromatography of diffusible GA from R_F 0.1 to 0.2 and extractable GA from R_F 0.2 to 0.3 side by side on one plate yielded GA activity in the same zone. Similar results were obtained when diffusible GA from R_F 0.3 to 0.4 was cochromatographed with the extractable GA from R_F 0.4 to 0.5.

In *Silene* extracts the GA activity at R_F 0.2 to 0.3 was always much higher than at R_F 0.4 to 0.5 (Fig. 1) when tested in the d5 corn assay. By contrast, in diffusates the GA activity on SD was approximately the same in both zones, and in some cases the activity at R_F 0.3 to 0.4 was even higher than at R_F 0.1 to 0.2 (Fig. 2). After 4 to 6 LD there was some increase in the GA activity in both zones, but with a somewhat larger increase at R_F 0.1 to 0.2 than at R_F 0.3 to 0.4.

A comparison was made of the amounts of GA obtained by diffusion from shoot tips and from mature leaves (Table VII). In agreement with the data in Table VI, LD treatment caused a substantial increase in the amount of diffusible GA obtained from shoot tips. However, little or no diffusible GA was obtained from the mature leaves regardless of the photoperiod. When the extractable GA obtained after the completion of the diffusion period is also considered, it is clear that under LD there is a substantial increase in the GA content of the shoot tips (including very young leaves), but an apparent decrease in the level of GA in mature leaves.

Influence of AMO 1618 on Flowering and Stem Elongation. As shown in Table VIII, control plants responded to LD treatment by 100% flowering and considerable stem elongation. By contrast, in plants treated with AMO 1618 there was essentially no stem growth, but the formation of flower primordia was not delayed. Thus LD treatment induced flowering in the absence of any significant stem elongation.

Influence of AMO 1618 on Endogenous GAs. When plants were treated with AMO 1618 for 14 SD, there was a 75 to 80% decrease in the level of extractable GA (Table IX). If plants were then moved to LD, the GA activity in treated plants was reduced

Table III. *Influence of Applied Gibberellin on Stem Elongation and Flowering on Short Days*

Gibberellin was applied as a 0.05-ml drop to the shoot apex on alternate days.

Experiment	GA Treatment		No. of Applications	Total Dosage	No. of Days after Start of Experiment: Plants Examined	Stem Height	Flowering[1]
	GA_3	GA_7					
	μg/plant			*μg*		*cm*	
1	Long day control		...	...	50	44.7 ± 1.2	4/4
	...	...	...	...	59	4.0 ± 0.2	0/4
	3		15	45	59	27.5 ± 2.0	0/4
	10		15	150	59	42.4 ± 2.2	0/4
		3	15	45	59	27.6 ± 2.2	0/4
		10	15	150	59	37.0 ± 3.3	1/4
2	Long day control		...	...	56	44.8 ± 5.3	4/4
	...	...	...	...	0	1.0 ± 0.1	0/4
	...	...	...	...	70	5.2 ± 0.4	0/7
	1		15	15	70	51.2 ± 11.9	4/8
	3		15	45	70	55.4 ± 7.8	2/8
	10		15	150	70	89.7 ± 13.7	6/8
		3	15	45	70	67.8 ± 11.5	5/7
		10	15	150	70	62.1 ± 12.1	4/8
		10	3	30	70	8.8 ± 1.1	0/8
		10	6	60	70	15.2 ± 1.1	0/8
		10	9	90	70	37.3 ± 10.0	2/8
		10	12	120	70	62.5 ± 7.7	4/7
		10	3	30	6	2.7 ± 0.1	0/8
		10	6	60	12	6.2 ± 0.2	0/8
		10	9	90	18	13.2 ± 0.7	0/8
		10	12	120	26	29.3 ± 2.0	1/8
3	...	...	...	...	60	3.7 ± 0.2	0/6
	0.001		10	0.01	60	4.7 ± 0.4	0/6
	0.01		10	0.1	60	4.4 ± 0.4	0/6
	0.1		10	1.0	60	11.4 ± 4.1	1/6
	1.0		10	10.0	60	36.6 ± 11.5	2/6
	10.0		10	100.0	60	58.2 ± 7.2	5/6

[1] No. of plants flowering/No. of plants examined.

even further to the point where it was below the limits of detection by the d5 corn bioassay (Fig. 3, Table IX). Likewise, treatment with AMO 1618 reduced the amount of GA obtained by diffusion from several hundred shoot tips below the limits of sensitivity of the d5 corn bioassay (Table X). The amount of GA extracted from shoot tips after completion of the diffusion period was also too low to measure. Thus after treatment with AMO 1618, which blocks stem elongation but has no effect on flowering, it is practically impossible to detect significant GA activity either in diffusates from several hundred shoot tips (Table X), or in extracts of 20 to 30 g dry weight of plant material (Table IX).

Comparison of Silene GAs with Authentic GAs. Extracts were chromatographed in solvent system 1 to give Silene I and II. Upon rechromatography of Silene I in solvent system 2, which separates GA_1 from GA_3, only one zone with biological activity was obtained. The R_F value was similar to that of GA_3 but was clearly different from that of GA_1 (Table XI). Silene I extracted from induced and vegetative material behaved identically in this solvent system.

When Silene II was rechromatographed in solvent system 3, which effectively separates GA_4, GA_5, and GA_7, two zones with biological activity were obtained (Table XII). One zone cochromatographed with GA_5 and could thus be identical with this GA or the closely related GA_{20} (28). The second zone had an R_F value which was consistently lower than that of GA_7. Again no differences were observed between extracts from induced and vegetative plants. On the basis of these results it appears, therefore, that floral induction of *Silene* is not accompanied by any qualitative changes in the endogenous GAs obtained by extraction.

Comparison of Extractable and Diffusible GAs. Authentic GAs were cochromatographed in solvent system 3 with acidic fractions prepared (*a*) from shoot tips prior to diffusion, (*b*) from shoot tips following a 24-hr diffusion period, and (*c*) from diffusates. In agreement with the data in Tables XI and XII, extracts of shoots showed three zones with GA activity (Fig. 4): one zone cochromatographed with GA_3, a second zone cochromatographed with GA_5, and a third zone ran slightly behind GA_7. On the other hand, only two zones with GA activity were detected in diffusates, the GA_5-like material being absent (Fig. 5). A similar pattern was observed in extracts prepared from shoot tips following the 24-hr diffusion period (Fig. 6).

Response of Plants on SD and LD to Applied GA. The possibility that plants are more sensitive to GA on LD than on SD was investigated (Table XIII). Plants treated with AMO 1618 under LD showed no significant stem elongation over the SD controls. However, plants which also received GA_3 did elongate, and, for a given concentration of GA_3, there was significantly more elongation on LD than on SD. For example, in plants treated with AMO 1618, 0.1 μg of GA_3 on LD caused as much stem elongation as did 3 μg of GA_3 on SD. Thus, it appears that, with respect to stem elongation, plants on LD are more sensitive to GA than plants on SD.

Table IV. *Influence of Photoperiod on Levels of Extractable Gibberellins*

Experiment	No. of Long Days	Gibberellin Activity
		μg GA_3 eq/100 g dry wt
1	0	1.09
	1	1.26
	2	2.18
	12	0.80
2	0	0.78
	4	2.31
	12	1.59
3	0	0.61
	2	0.51
	5	0.48
	12	0.55
4	0	1.62
	4	2.30

Table V. *Influence of Photoperiod on Levels of Extractable Gibberellins Obtained from Different Parts of the Plant*

No. of Long Days	Gibberellin Activity	
	Shoot tips	Leaves and Stems
	μg GA_3 eq/100 g dry wt	
0	3.13	0.38
12	5.25	0.51

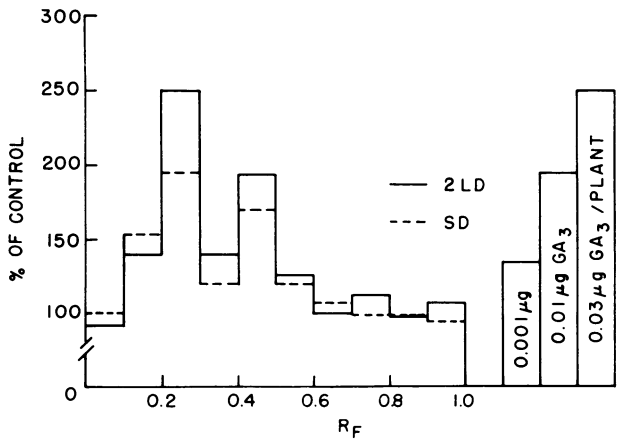

FIG. 1. Comparison of the gibberellin activity as measured in the d5 corn assay of extracts prepared from *Silene* plants on short days or after exposure to 2 long days. Thin layer chromatography in solvent system 1.

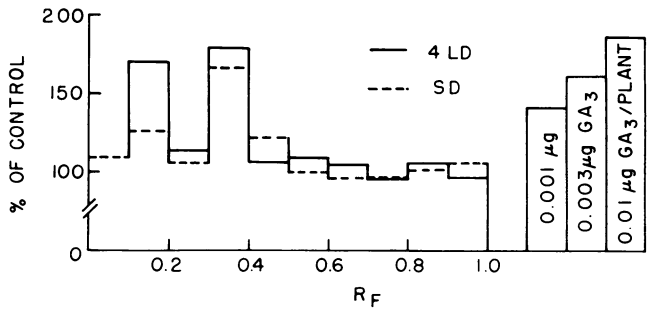

FIG. 2. Comparison of gibberellin activity obtained by diffusion from shoot tips of *Silene* plants on short days or after 4 long days.

Table VI. *Influence of Photoperiodic Treatment on Diffusible Gibberellins Obtained from Shoot Tips*

Experiment	Photoperiodic Treatment of Plants prior to Diffusion	Gibberellin Activity	
		Diffusate	Shoot tips after diffusion
		μg GA_3 eq/100 g dry wt	
1	SD	0.60	2.40
	2 LD	0.87	2.40
	4 LD	6.39	4.18
	10 LD	0.29	7.00
2	SD	0.18	0.91
	2 LD	0.62	0.98
	4 LD	0.46	1.13
	6 LD	0.91	2.86
	8 LD	0.26	1.46
3	SD	0.32	2.58
	2 LD	0.83	2.31
	4 LD	1.12	3.27
	5 LD	2.18	4.15
	6 LD	3.17	2.43
	10 LD	1.14	3.97

Table VII. *Influence of Photoperiod on Diffusible Gibberellins Obtained from Shoot Tips or Mature Leaves*

Treatment	Gibberellin Activity	
	Short day	Four long days
	μg GA_3 eq/100 g dry wt	
Diffusate from shoot tips	0.84	1.96
Shoot tips after diffusion	2.35	4.17
Diffusate from leaves	0.10	<0.09
Leaves after diffusion	1.01	0.41

DISCUSSION

In the present study both GA_3 and GA_7 caused flowering in plants on SD in contrast to previous reports that GA_3 was either ineffective (26) or effective only at very high doses (40). Furthermore, in the work of Michniewicz and Lang (26) a minimal total dose of 150 μg of GA_7 was required for flower formation while in the present study 1 to 10 μg of GA_3 were sufficient to cause some flowering. The reason for these discrepancies is not clear. However, it should be noted that in previous studies (39, 40) a minimum of 4 to 7 LD was needed to induce some flowering, while in the present study 2 LD gave some flowering and 4 LD gave essentially 100% flowering. Thus the present plant material exhibits an increased sensitivity to applied GA. Wellensiek (42) has shown that it is possible to select from the original 'N' strain of Liverman several "substrains" which differ in their photoperiodic sensitivity. One of these substrains appears more similar in its response to LD to the material used in the present study than to that used in earlier work. Thus it appears that in the production of the present seed batch there was an unconscious selection for plants with increased sensitivity to LD which resulted in

Table VIII. *Progress of Stem Elongation and Flowering in the Presence or Absence of AMO 1618*

Plants received 10 ml of 10^{-2} M AMO 1618 or H_2O on alternate days for the duration of the experiment.

Photoperiodic Treatment	Stem Height		Flowering[1]	
	−AMO	+AMO	−AMO	+AMO
	cm			
Zero time	2.2 ± 0.2		0/4	
10 SD-6 LD	2.4 ± 0.2	2.5 ± 0.2	3/7	2/7
10 SD-9 LD	4.5 ± 0.7	2.5 ± 0.1	6/7	6/7
10 SD-12 LD	4.5 ± 0.5	2.7 ± 0.1	7/7	6/7
10 SD-12 LD-3 SD	7.0 ± 0.7	3.1 ± 0.3	7/7	7/7
10 SD-12 LD-6 SD		3.3 ± 0.5		7/7
10 SD-12 LD-12 SD	27.6 ± 1.5	3.5 ± 0.4	7/7	7/7
10 SD-12 SD-12 SD	3.3 ± 0.4		0/7	

[1] No. of plants flowering/No. of plants examined.

Table IX. *Influence of AMO 1618 on Levels of Extractable Gibberellins Obtained with Different Photoperiodic Treatments*

Plants received 10 ml of 10^{-2} M AMO 1618 or H_2O on alternate days for the duration of the experiment.

Experiment	Photoperiodic Treatment	AMO 1618	Gibberellin Activity
			μg GA_3 eq/ 100 g dry wt
1	14 SD	−	0.29
	14 SD	+	0.05
	14 SD-12 LD	−	0.22
	14 SD-12 LD	+	<0.04
2	14 SD	−	0.25
	14 SD	+	0.06
	14 SD-1 LD	−	0.51
	14 SD-1 LD	+	0.05
	14 SD-3 LD	−	0.29
	14 SD-3 LD	+	<0.05

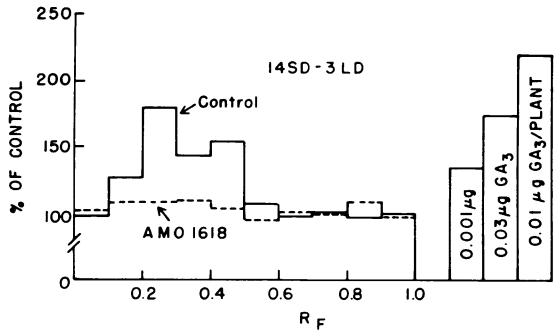

FIG. 3. Effect of AMO 1618 on gibberellin activity extracted from *Silene*. AMO 1618 treatment given during 14 short days, followed by 3 long days.

the present plant material having an increased sensitivity to both LD and applied GA.

In rosette LDP flowering and stem elongation usually occur more or less simultaneously, and under normal conditions these two processes cannot be separated. However, the results of the present study show that stem elongation can occur in the absence

Table X. *Influence of AMO 1618 on Diffusible Gibberellins Obtained after Different Photoperiodic Treatments*

Plants received 10 ml of 10^{-2} M AMO 1618 or H_2O on alternate days for the duration of the experiment.

Photoperiodic Treatment of Plants prior to Diffusion	AMO 1618	Gibberellin Activity	
		Diffusate	Shoot tips after diffusion
		μg GA_3 eq/100 g dry wt	
14 SD	−	0.24	0.57
14 SD	+	<0.10	<0.10
14 SD-4 LD	−	0.44	0.76
14 SD-4 LD	+	<0.10	<0.10

Table XI. *Cochromatography of Silene I with GA_1 and GA_3 in Solvent System 2*

Plates developed to 30 cm.

No. of Long Days	R_F Values		
	Silene I	GA_1	GA_3
0	0.34	0.38	0.33
6	0.31	0.36	0.31

Table XII. *Cochromatography of Silene II with GA_4, GA_5, and GA_7 in Solvent System 3*

Plates developed to 15 cm.

No. of Long Days	R_F Values				
	Silene II		GA_4	GA_5	GA_7
0	0.30	0.59	0.76	0.29	0.66
6	0.24	0.43	0.61	0.25	0.48

of flowering (GA application on SD), or flowering can occur in the absence of stem elongation (AMO 1618 treatment on LD). Similar results have been obtained in *Samolus* where treatment on LD with CCC causes complete inhibition of stem elongation but only slight inhibition of flowering (1). In addition, in *Hyoscyamus* some stem elongation was obtained in the absence of any flowering under suboptimal photoperiods at 28 C (22), while in *Rudbeckia* under certain special conditions some flowering could be obtained in the virtual absence of any stem elongation (27). Thus it is clear that although flowering and stem elongation are closely connected under normal conditions in rosette LDP they can be experimentally separated and thus represent independent developmental processes.

The basic question concerning the induction of flowering in *Silene* by applied GA is whether this response reflects a physiological function of GA, or only a pharmacological effect. That is, are endogenous GAs limiting for flowering under SD conditions? If this were the case, one might expect that plants exposed to LD would show a substantial increase in the GA content or the appearance of some new GA after 2 or 3 LD (minimum needed to induce flowering), but prior to the onset of flower initiation (about the 6th day). It is important to emphasize that changes in endogenous GAs that occur after the beginning of flower formation, such as have been reported before (7, 25), may be important for flower development. However, only those changes due to photoinduction which can be detected prior to the onset of flower

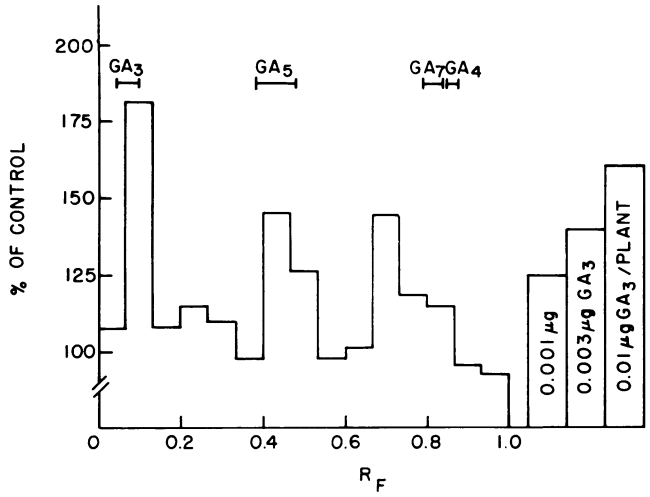

FIG. 4. Cochromatography of authentic gibberellins with gibberellins extracted from shoot tips of *Silene* plants that had received 2 long days. Thin layer chromatography in solvent system 3.

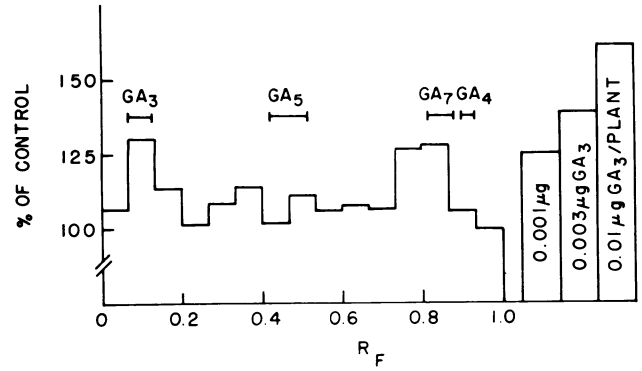

FIG. 5. Cochromatography of authentic gibberellins with gibberellins obtained by diffusion from shoot tips of *Silene* plants that had received 2 long days.

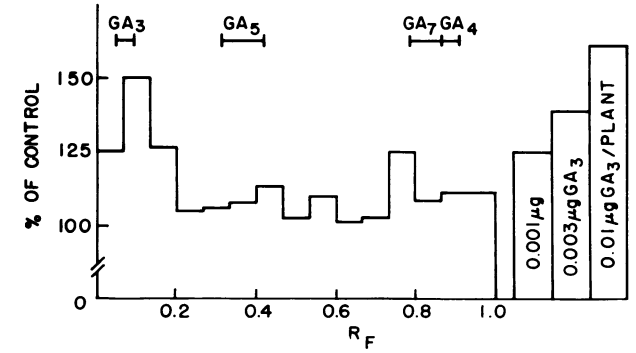

FIG. 6. Cochromatography of authentic gibberellins with gibberellins extracted from *Silene* shoot tips following a 24-hr diffusion period. The shoot tips were obtained from plants that had received 2 long days.

formation should be considered for a possible role in the regulation of flower initiation.

Although the native GAs of *Silene* have not been chemically identified, evidence obtained by means of thin layer chromatography in three different solvent systems strongly suggests that there are no qualitative changes in the patterns of extractable or diffusible GAs when plants are moved from SD to LD (Tables XI and XII; Figs. 1 and 2). However, the over-all level of extractable GA does increase somewhat over the SD control after 2 or more LD (Table IV), while the amount of diffusible GA obtained from shoot tips increases several times after exposure to 4 to 6 LD (Table VI). If this increase in GA is important for flower formation, it should be possible by substantially reducing the level of endogenous GA to prevent, or at least delay flowering. However, treatment with AMO 1618 sufficient to block stem elongation completely and reduce the level of extractable and diffusible GA below the limits of detection in the d5 corn assay (Tables IX and X) had no effect at all on flower initiation (Table VIII). It could still be argued that only trace amounts of GAs are needed to induce flowering. But this argument is difficult to accept since plants on SD had many-fold higher GA levels than did the plants treated with AMO 1618 on LD, yet the former remained vegetative while the latter flowered. Attempts to relate flowering to bound GAs which can be converted to the free form by mild acid hydrolysis (2) were negative since no bound GA could be detected in *Silene* grown on either SD or LD. Therefore, these results indicate that in *Silene* the flowering process is largely, if not entirely, independent of GA control.

The apparent independence of flowering in *Silene* from GA suggests that the induction of flowering by applied GA is a pharmacological rather than a physiological effect. Further support for this conclusion comes from the observation that the flowering response on SD due to applied GA is quite variable, even when plants receive a total dose of 150 μg of GA_3 (Table III). By contrast, flowering is always 100% if the plants receive 4 or more LD. Furthermore, when *Silene* plants are moved from SD to LD, microscopically detectable flower primordia are first seen just prior to the onset of measurable stem elongation (Tables II and VIII). By contrast, when plants are treated with GA on SD, they first respond by rapid stem elongation and only later

Table XIII. *Influence of GA_3 on Flowering and Stem Elongation on Short and Long Days in the Presence or Absence of AMO 1618*

Plants received 14 preliminary short days followed by short or long day treatment for 7 weeks. Plants received 10 ml of 10^{-2} M AMO 1618 or H_2O on alternate days during 14 preliminary short days and 10 ml of 10^{-3} M AMO 1618 or H_2O on alternate days for the rest of experiment. GA_3 treatments started on day 15 and were applied as 0.05-ml drops to the stem apex on alternate days for a total of 14 applications.

Photoperiod	AMO	GA_3	Stem Height	Flowering[1]
		μg/plant	*cm*	
SD	−	...	3.4 ± 0.2	0/7
LD	−	...	45.0 ± 2.6	7/7
SD	+	...	2.5 ± 0.2	0/7
SD	+	0.3	6.2 ± 1.9	0/7
SD	+	1.0	13.1 ± 2.3	0/7
SD	+	3.0	16.9 ± 2.4	0/7
LD	+	...	4.7 ± 1.5	5/6
LD	+	0.1	16.0 ± 3.7	7/7
LD	+	0.3	25.9 ± 1.9	5/5
LD	+	1.0	27.2 ± 5.4	7/7
LD	+	3.0	30.6 ± 3.0	7/7
SD	−	...	3.4 ± 0.2	0/7
SD	−	0.3	9.3 ± 1.0	0/5
SD	−	1.0	18.9 ± 2.2	0/5
SD	−	3.0	23.1 ± 3.8	0/5

[1] No. of plants flowering/No. of plants examined.

are flower primordia formed, if they are formed at all (Table III). Thus it seems clear that the effect of applied GA on flowering is indirect, with the primary effect being to stimulate stem elongation. A similar conclusion has been reached for the rosette LDP *Rudbeckia* where it was shown that GA acts at the shoot apex to stimulate stem elongation and has only an indirect effect on flowering (5, 6). Furthermore, application of CCC to radish inhibited stem elongation and reduced the GA content without any inhibitory effect on flowering (36). Thus in certain plants including *Silene*, flowering appears to be largely if not entirely independent of GA action. However, in other plants such as *Bryophyllum* (47), *Lemna* (9), *Pharbitis* (44), and *Samolus* (1), a clear dependence upon GA for flowering can be demonstrated. In *Bryophyllum* (47) GA is required for production of the floral stimulus in the leaf, whereas in the SDP *Pharbitis* GA seems to be necessary for the floral stimulus to exert its action in the apex (44). Even in these cases, however, it is not clear whether GA has a direct effect on the flowering process itself, or only an indirect effect which enables the flowering process to proceed to completion. Thus the degree to which the flowering process is influenced by GA action varies considerably from a clear dependence in plants such as *Bryophyllum* to an apparent independence in plants such as *Silene*.

Unlike flowering, stem growth in *Silene* is clearly dependent on the presence of GA. Applied GAs cause stem elongation under SD (Table III), while reducing the level of endogenous GA by treatment with AMO 1618 under LD suppresses stem growth (Table VIII). However, it is puzzling that plants on SD grow as rosettes while those on LD exhibit rapid stem elongation when there is only a small difference in the GA content of plants on SD and LD as measured by extraction. The following points may explain this discrepancy.

First of all, GA extractions provide only a static picture of the endogenous GAs since they give the GA content at the time of metabolic killing but tell nothing about the rates of GA biosynthesis and metabolism. If one accepts the idea that the amount of GA diffused into agar blocks is a measure of GA production (16, 17), it follows that in *Silene* production of GA occurs in the shoot tips, including young leaves, but not in mature leaves (Tables VI and VII). Furthermore, exposure to 4 to 6 LD increases GA production up to 10-fold. This increase occurs only in the region of the shoot apex just prior to the onset of stem elongation, suggesting that it may be an important factor in the initial stimulation of stem growth. Since GA levels in extracts do not increase over the SD control by more than a factor of 2, it must be concluded that increased GA biosynthesis under LD is accompanied by increased GA utilization.

Secondly, the data in Table XIII indicate that *Silene* plants under LD conditions respond with more stem growth to a given dose of applied GA_3 than do plants under SD. It seems reasonable to assume that this increased sensitivity to GA is at least partially responsible for the stem elongation which occurs on LD. However, it is not known at present how soon after the transfer from SD to LD this increased sensitivity becomes established, and thus the extent to which this change influences stem growth remains to be determined.

A reduction in the growth response of dwarf peas to GA due to light has been described by Kende and Lang (18), and in more detail by Musgrave and Kende (29). It should be noted, however that in peas light decreases the response to GA, whereas in *Silene* long photoperiods increase the response.

A comparison of GA-like activity obtained by extraction and by diffusion points up some interesting differences. In extracts the level of GA_3-like activity was much higher than the GA activity with an R_F slightly less than that of GA_7 (Fig. 4). In addition, GA_5-like activity was detected in extracts of fresh plant material. However, in extracts of shoot tips following the diffusion period the level of GA_5-like activity was very low and in some experiments appeared to be absent (Fig. 6). Possibly the decrease of the GA_5-like activity during diffusion indicates that this GA is metabolized more rapidly than others. When GA was obtained by diffusion instead of extraction, the level of GA_3-like activity was approximately equal to the GA activity with an R_F close to GA_7. Furthermore, GA_5-like activity was never detected in diffusates. Analogous results have been reported for peas where both GA_1- and GA_5-like activity were obtained by extraction, but in diffusates only the GA_1-like activity was detected (15).

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