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EFFECT OF NINE DIFFERENT GIBBERELLINS ON STEM ELONGATION AND FLOWER FORMATION IN COLD-REQUIRING AND PHOTOPERIODIC PLANTS GROWN UNDER NON-INDUCTIVE CONDITIONS*

By

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With 2 Figures in the Text

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Treatment with gibberellin causes flower formation in numerous plants under conditions in which they would normally remain vegetative or would flower only with considerable delay (for recent reviews, see LANG and REINHARD 1961; LANG 1961). The plants in which gibberellin has this effect are rosette plants which normally flower upon exposure to a period of low temperatures, or to long days, or both. Gibberellin also causes flower formation in certain long-short-day plants if they are maintained on short but not on long days, *i. e.*, it "substitutes" for the long-day but not for the short-day part of their dual induction requirement.

At present, gibberellin is still the only known chemical substance which is capable of causing flower formation under non-inductive conditions and in a large number of different species belonging to definite physiological classes of plants. The exposure of cold-requiring and long-day plants to their normal inductive conditions is accompanied by profound, quantitative as well as qualitative changes in the native or endogenous gibberellins of these plants (HARADA and NITSCH 1959; LANG 1960; CHAJLAKHIAN and LOZHNIKOVA 1960; REINHARD and LANG 1961). Recent results of ZEEVAART and LANG (1962) with the long-short-day plant *Bryophyllum daigremontianum* indicate, although indirectly, that the transfer from long to short days, which is necessary for flower induction, also causes an increase in the endogenous gibberellin level in the plants. It is thus probable that endogenous gibberellins participate in the regulation of flower formation, although the effect may be indirect, being primarily concerned with stem elongation.

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However, gibberellin application does not result in flower formation in all cold-requiring and long-day rosette species kept under strict noninductive temperature or photoperiod regimes. In some cases, it is effective only under threshold conditions under which other factors, *e. g.* applied auxin (LIVERMAN and LANG 1956), also may promote flower formation; in others, it causes stem elongation but no flower initiation or is without effect on either process.

One of the reasons which has been suggested to account for these negative results was the use of a "wrong" gibberellin. Almost all studies on the flower-inducing action of gibberellin were made with gibberellic acid (=gibberellin A_3), a few with mixtures of this gibberellin and gibberellin A_1. BUKOVAC and WITTWER (1958, 1961) compared the effectiveness of gibberellins A_1, A_2, A_3 and A_4 on flower formation in lettuce and dill and found A_1 and A_3 highly, A_2 and A_4 little active. At present, however, the number of chemically identified gibberellins has increased to nine, mainly thanks to research efforts at the Akers Laboratories, Imperial Chemical Industries, Ltd. (The Frythe, Welwyn, Herts, England). Five of these gibberellins (A_2 through A_4, A_7, and A_9) were isolated from the fungus *Fusarium moniliforme*; three (A_5, A_6, A_8) from immature seeds of *Phaseolus multiflorus*; one (A_1) was found in either source (see CROSS *et al.* 1961; MACMILLAN *et al.* 1961).

The physiological properties of these gibberellins are different. For example, BRIAN *et al.* (1962) showed that gibberellin A_9 is highly active in promoting leaf sheath elongation in the *d*-3 mutant of maize, less active in the *d*-5 mutant and of very low activity in the *d*-1 mutant and also in stem elongation of dwarf Meteor peas. In certain systems, gibberellic acid is less active than other gibberellins. This is particularly true in cucurbits in which growth promotion by gibberellin A_4 (LOCKHART and DEAL 1960; BRIAN and HEMMING 1961; BUKOVAC and WITTWER 1961; HALEVY and CATHEY 1961) and gibberellins A_7 and A_9 (BRIAN and HEMMING 1961; BRIAN *et al.* 1962) is considerably superior to that by gibberellic acid. HASHIMOTO and YAMAKI (1959, 1960) reported that gibberellin A_4 was also more effective than A_3, A_1 and A_2 in promoting dark germination in tobacco seeds and expansion of bean and radish leaf discs. Gibberellins A_5, A_7 and A_9 were more active than A_3 in the *d*-5 dwarf mutant of maize (BRIAN *et al.* 1962).

It seemed therefore possible that gibberellic acid might not be the most efficient gibberellin to induce flower formation in all cold-requiring and long-day rosette plants and that in at least some of those species which did not form flowers after gibberellic-acid treatment positive responses might be obtained with other gibberellins.

The present study was undertaken to test this possibility. Five plants were included:

a) A biennial strain of *Centaurium minus* MOENCH, a cold-requiring plant which exhibits a ready flowering response upon gibberellic-acid treatment (CARR, MCCOMB and OSBORNE 1957);

b) *Myosotis alpestris* L., a cold-requiring plant in which gibberellic-acid treatment does not cause flower formation (personal communication, Professor S. J. WELLENSIEK, Laboratory of Horticulture, Agricultural University, Wageningen, The Netherlands);

c) *Crepis parviflora* DESF., a long-day plant with ready flowering response to gibberellic-acid treatment (Dr. M. NEGBI, unpublished observations);

d) *Silene armeria* L., a long-day plant in which gibberellic acid causes flower formation only after prolonged application of massive doses (LANG 1957);

e) *Bryophyllum crenatum* BAK., a long-short-day plant with ready flowering response to gibberellic-acid treatment under short-day conditions (HARDER and BÜNSOW 1956; PENNER 1960).

Centaurium and *Myosotis* were grown under non-thermoinductive temperatures and long-day conditions, *Crepis*, *Silene* and *Bryophyllum* on short days.

Because of the scarcity of the pure gibberellins, except gibberellic acid, the scope of the tests had to be limited, both with respect to the number of plants per treatment and the dosage range. Three to five plants per treatment and four dosage levels (0.3, 1.0, 3.0 and 10.0 μg per plant per application) were used, except with gibberellic acid which was also applied at 30 μg and 100 μg. The quantitative data on stem growth must therefore be considered as preliminary, but the results concerning flower formation were so clear-cut as to justify publication and certain conclusions. The principal results with *Myosotis* have been published in a preliminary note (MICHNIEWICZ and LANG 1962).

We are deeply indebted to the above-named Imperial Chemical Industries laboratory for supplies of the gibberellins A_1 and A_5 through A_9; to Professor Y. SUMIKI, University of Tokyo, Japan, for repeated supplies of gibberellins A_2 and A_4; and to Abbott Research Laboratories (Scientific Division), North Chicago, Illinois, U.S.A., for supplies of gibberellin A_1. The gibberellic acid used was a preparation from Merck and Co., Rahway, N.J., U.S.A.

Material and Methods

The plants used have been listed above. The *Centaurium minus* seeds were received from Professor D. J. CARR, School of Botany, University of Sydney, Australia (presently, Department of Botany, Queen's University, Belfast, North Ireland), those of *Myosotis alpestris* from Professor WELLENSIEK. The *Crepis parviflora* strain came from the collection of the late Professor E. B. BABCOCK, University of California, Berkeley, and was supplied by the Department of Genetics

of this school. We greatly appreciate the kindness of these individuals and institutions. The *Silene armeria* strain was the same as used in earlier work (LANG 1957), the *Bryophyllum crenatum* was a strain of unknown origin and maintained locally by vegetative propagation.

The experiments were done under the controlled conditions of the Earhart Plant Research Laboratory. *Crepis* and *Myosotis* were grown in soil, the other 3 species in a gravel-vermiculite mixture; all were watered with half-strength complete Hoagland nutrient solution as needed. The plants were raised and treated under the following daily conditions:

Centaurium, Myosotis — 16 hours of light (natural day *plus* incandescent light from 06:00 to 08:00 and 16:00 to 22:00), temperature 23° from 08:00 to 17:00 and 19° from 17:00 to 08:00;

Bryophyllum, Silene — 8 hours of natural light (08:00 to 16:00) at 23°, 16 hours of dark at 15°;

Crepis — 6 hours of natural light (08:00 to 14:00) at 23°, 18 hours of dark at 15°.

The gibberellin solutions were prepared in 0.05 per cent of Polyglycol 31 (Dow Chemical Corp., Seal Beach, California, U.S.A.) as wetting agent and applied to the tips of the plants always in a quantity of 50 μl. The *Bryophyllum* plants were treated five times, the others ten to fifteen times in intervals between 2 and 4 days; the precise schedules will be given with the description of the individual experiments. Plants which did not show a visible flowering response were continued for several weeks after the end of the gibberellin treatment and then examined under a dissecting microscope. Control plants which were maintained in all experiments remained strictly vegetative.

In the following text and tables, the nine gibberellins will be abbreviated as GA_1, GA_2, *etc.*

Results

1. Myosotis alpestris. The plants were seeded on May 25, 1961, the gibberellin treatment started on August 15. Fifteen applications were made, the first 10 every other day, the last five every third. Five plants were used per treatment, except with GA_3 where ten were used. The results are summarized in Table 1; an illustration of plants treated at the two higher levels was published in MICHNIEWICZ and LANG (1962).

Flowering occurred in the two highest GA_7 treatments and the highest GA_1 treatment. Greatest stem elongation was caused by treatment with GA_7, followed by GA_1, GA_3, GA_4 and GA_5; GA_6 and GA_9 being next and GA_2 and GA_8 last.

Flower induction by gibberellin treatment was associated with strong stem elongation. However, it should be noted that the correlation was not complete. Thus, GA_3, GA_4, and GA_5 caused about as much stem elongation as GA_7 but did not cause any flower formation. Also, GA_3 at 30 μg and 100 μg per application caused more elongation than GA_7 at 3 μg and 10μ g (and considerably more than GA_1 at 10 μg) but did not result in flower formation.

2. Centaurium minus (biennial strain). Plants seeded April 21, 1961; treatment started October 7. The first seven treatments were admin-

Table 1. *Effect of gibberellins A_1 through A_9 on stem elongation and flower formation in non-thermoinduced Myosotis alpestris*

GA per application (μg)	GA_1	GA_2	GA_3	GA_4	GA_5	GA_6	GA_7	GA_8	GA_9
0.3	110	R	130	141	119	R	173	R	R
1	120	R	152	159	130	R	190	R	110
3	182	R	166	180	159	111	*265*	R	131
10	*210*	105	241	221	238	180	**290**	80	198
30	—	—	308	—	—	—	—	—	—
100	—	—	350	—	—	—	—	—	—

Figures in italics = part of plants flowering. **Figures in boldface** = all plants flowering. R = rosette stage.

Figures indicate height of stems (in flowering plants, without inflorescence) in mm, measured 3 months after start of treatment.

Table 2. *Effect of gibberellins A_1 through A_9 on stem elongation and flower formation in non-thermoinduced Centaurium minus*

GA per application (μg)	GA_1	GA_2	GA_3	GA_4	GA_5	GA_6	GA_7	GA_8	GA_9
0.3	R	R	*160*[2a]	35	20	R	20	R	R
1	20	R	*300*[2a]	*350*[2]	50	R	60	R	25
3	*260*	20	v[1]	F[3]	*250*[2]	30	**230**	R	*230*[2]
10	**380**	60	F[3]	**340**[3a]	F[3]	*230*[2a]	F[3]	R	F[3]
30	—	—	F[3]	—	—	—	—	—	—
100	—	—	d	—	—	—	—	—	—

[1] Main shoots dead after some elongation; lateral shoots reverted to rosette growth.

[2] One plant flowering; others have elongated stems but in vegetative state.

[2a] One plant flowering; in others, main shoots dead, in vegetative state.

[3] Main shoots dead after some elongation; microscopic flower buds on lateral shoots.

[3a] One plant flowering; in others main shoots dead, floral primordia in lateral shoots.

Figures in italics = part of plants flowering. **Figures in boldface** and **F** = all plants flowering. R = rosette stage. v = plants elongated but vegetative. d = plants dead.

Figures indicate height of stems (intact plants only) in mm, $9^1/_2$ weeks after start of treatment.

istered on alternate days, the next 8—10 every third day, the last two every fourth. Three plants were used per variant.

The results with this plant were somewhat vitiated by the fact that the main shoots of many individuals died at various times during or after the treatment. Differences in the activity of the nine gibberellins in flower formation were, however, quite marked. It appears that GA_3 has here the greatest flower-inducing effect, followed by GA_1, GA_4, GA_5,

GA_7 and GA_9 (GA_4 and GA_7 being perhaps somewhat more effective than the other three), and lastly by GA_6. GA_2 caused only some stem elongation (at the two higher dosage levels); GA_8 caused neither flower initiation nor stem elongation. The data on stem length are too incomplete to permit valid conclusions concerning correlations between effects on stem elongation and flower formation, except that a substantial degree of the former seems always to be associated with the latter.

3. Silene armeria. Plants seeded May 25, 1961; start of gibberellin treatment August 1. Treatment schedule as in *Myosotis* (15 treatments; first ten every other, last five every third day); five plants per treatment, ten in the case of GA_3.

Table 3. *Effect of gibberellins A_1 through A_9 on stem elongation and flower formation in Silene armeria under short-day conditions*

GA per application (μg)	GA_1	GA_2	GA_3	GA_4	GA_5	GA_6	GA_7	GA_8	GA_9
0.3	85	R	90	61	85	85	136	R	90
1	150	R	196	133	237	158	271	R	150
3	171	R	224	250	246	210	306	R	252
10	352	80	440	475	318	388	**525**	144	305
30	—	—	480	—	—	—	—	—	—
100	—	—	505	—	—	—	—	—	—

Boldface figures = flowering. R = rosette stage.

Figures indicate height of stems in mm, 2-$^1/_2$ months after start of treatment.

The final height of the plants and the flowering response are shown in Table 3. Flower formation occurred in only one treatment, the highest dosage level of GA_7, *i. e.* 10 μg per application or a total of 150 μg per plant. GA_3 did not cause flower formation even at the tenfold level (100 μg per application; 1,500 μg total). In previous experiments (LANG 1957) the minimal levels of GA_3 necessary to induce a similar flowering response in *Silene* had been 20—50 μg per plant per treatment, applied for periods of approximately 3 months, *i. e.* total amounts between about 1,800 and 4,500 μg. In those experiments, the plants had received daily applications which are somewhat less effective than intermittent ones at the same total level (LANG, unpublished experiments with biennial *Hyoscyamus niger*). But even assuming that intermittent treatment would have reduced the minimal GA_3 level by one half, it is clear that the effectiveness of GA_7 with respect to flower induction in *Silene* exceeds that of GA_3 by a factor of about 5 or more.

With respect to their effect on stem elongation, the nine gibberellins can be roughly grouped into three groups: (1) GA_3, GA_4 and GA_7; (2) GA_1, GA_5, GA_6 and GA_9; (3) GA_2 and GA_8. This is made even

clearer by Fig. 1 which illustrates the course of stem elongation in plants treated at the 10 μg dosage level. The relative positions of the curves of the nine gibberellins remain essentially the same, except that the GA_1-treated plants appear to overtake the GA_5- and GA_9-treated ones after the treatment has been terminated. This would indicate that the effect of GA_1 is relatively longer-lasting, a point which may be worth some further study.

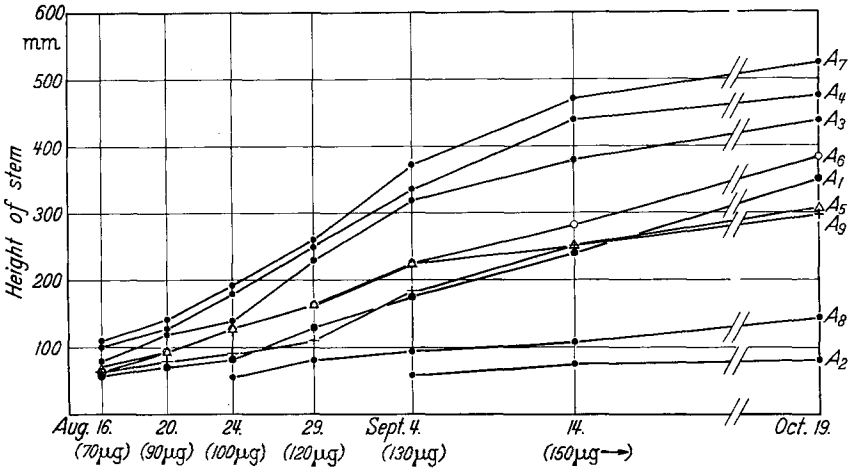

Fig. 1. Influence of gibberellin (10 μg per application) on stem elongation in the long-day plant *Silene armeria* grown under short-day conditions. Abscissa: dates of measurement and (in parentheses) amounts administered by these dates. The rosette axis of the plants was approximately 50 mm long

Flower formation occurred in the treatment which also resulted in maximal stem elongation. Nevertheless, the differences in the activity of the different gibberellins in this respect seems to be smaller than the differences in their flower-inducing effectiveness, for GA_4 at 10 μg per application and GA_3 at the 3- and 10fold dosage level caused almost as much stem elongation as GA_7 at 10 μg, but the plants showed no sign of flower induction.

4. Crepis parviflora. Plants seeded June 27, 1961; gibberellin treatment started October 7. Ten applications; first seven every other day, last three every third day; three plants per variant.

The results are summarized in Table 4. With the exception of GA_8, all gibberellins which were tested caused stem elongation and with the exception of GA_8 and GA_6 also unequivocal flower formation. Their activity showed, however, considerable gradation. Only GA_4 and GA_7 caused flower formation in all treated plants at the lowest dosage level used (0.3 μg per application). They were followed by GA_1 and GA_3 which reached full effectiveness at the second or third dosage levels (1.0 μg

and 3.0 μg), while GA_2, GA_5 and GA_9 were effective only at the highest level (10 μg).

With respect to stem elongation, *Crepis* differs from *Centaurium*, *Myosotis* and *Silene* in as far as almost any stem elongation seems to be associated with flower formation. Even those plants which exhibited some degree of elongation but are not listed as flowering in Table 4 (GA_1 at 0.3 μg per application; GA_3 at 0.3 μg and 1.0 μg; GA_5 at 3.0 μg; GA_6 at 10 μg) had markedly enlarged growing points which are characteristic of individuals in the early stages of inflorescence initiation. The

Table 4. *Effect of gibberellins A_1 through A_9 on stem elongation and flower formation in Crepis parviflora under short-day conditions*

GA per application (μg)	GA_1	GA_2	GA_3	GA_4	GA_5	GA_6	GA_7	GA_8	GA_9
0.3	60[1]	R	*75*	**120**	R	R	**55**	R	R
1	**83**	R	194[2]	**143**	R	R	**250**	R	R
3	**335**	R	**246**	**292**	50	R	**320**	R	R
10	**360**	**33**	**290**	**228**	**198**[1]	43[1]	**383**	R	**106**
30	—	—	**328**	—	—	—	—	—	—
100	—	—	**368**	—	—	—	—	—	—

[1] Only 1 plant had elongated stem and enlarged growing point.
[2] All plants had elongated stems and enlarged growing points.

Figures in italics = part of plants with inflorescence buds. **Figures in boldface** = all plants with inflorescence buds.

Figures indicate height of stems in mm, 2 months after start of gibberellin treatment.

degree of total elongation caused by the different gibberellins shows certain differences (see Table 4). For example, GA_4 seems to cause relatively more elongation than GA_1 and GA_7 at lower dosages but less at higher ones. However, a larger material would be required to ascertain whether such apparent differences in activity are consistent.

5. Bryophyllum crenatum. The plants used for the experiment were grown from cuttings, were approximately 6 months of age and had a minimum of 12 leaf pairs on the main shoots. Gibberellin applications were made on October 7, 9, 14, 19 and 28, 1961; three shoots were used in each variant[1]. *Bryophyllum crenatum* is very sensitive to gibberellin action with respect to flower induction; according to PENNER (1960), 0.15 μg of GA_3 are sufficient to cause flower formation in short-day-grown plants. Our material appeared to be somewhat less sensitive; preliminary

[1] In plants with several shoots, individual shoots were used for individual treatments. Preliminary trials showed that, at least at the levels employed in these experiments, there was no transfer of the gibberellin effect from a treated shoot to non-treated ones.

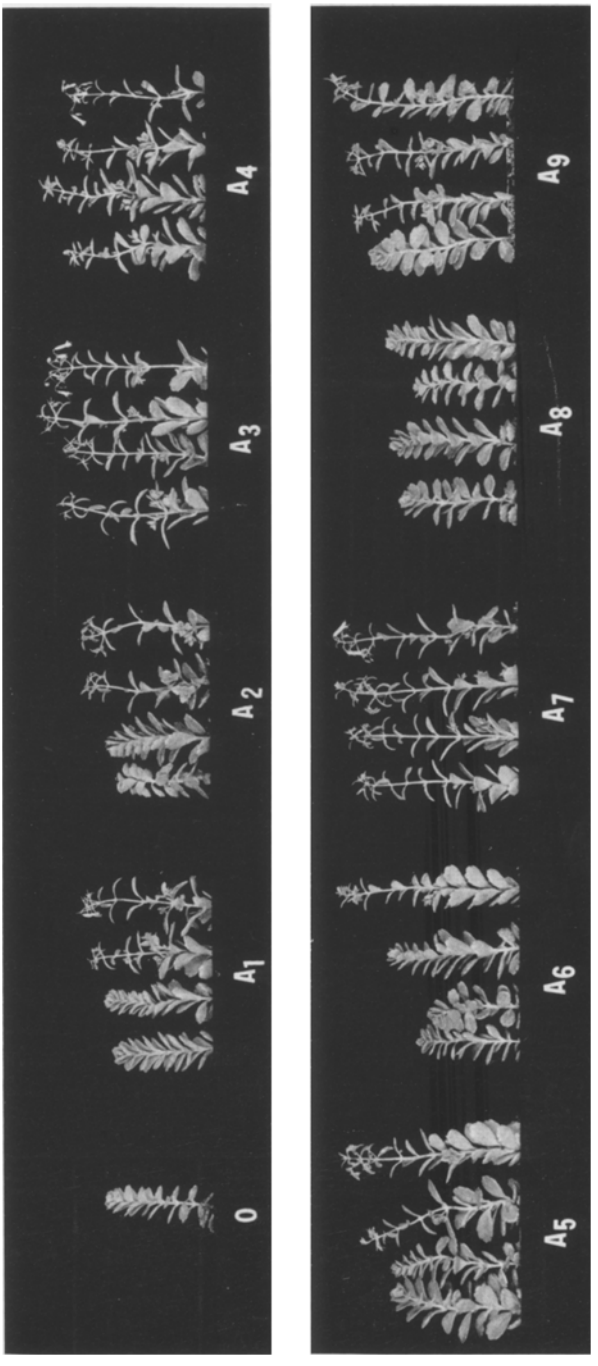

Fig. 2. Representative plants (upper stem portions) of *Bryophyllum crenatum* showing the effect of five applications of gibberellins A_1 through A_9. In each group, from left to right: plants treated with 0.3–1–3–10 μg per application. 0 = untreated control plant. Phot. J. W. McCLANAHAN

tests showed that about 1.5 μg GA_3 were required for a flowering response.

The results of the experiment with GA_1 through GA_9 are summarized in Table 5; representative plants from the different treatments are shown in Fig. 2. In decreasing order of activity with regard to flower induction the nine gibberellins can be grouped as follows: (1) GA_3, GA_4, GA_7 — active at the lowest level used; (2) GA_1, GA_2, GA_5, GA_9 — active at intermediate levels; (3) GA_6, GA_8 — active only at the highest level, or inactive.

Table 5. *The influence of gibberellins A_1 through A_9 on flower formation in Bryophyllum crenatum plants grown under short-day conditions*

GA per application (μg)	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
0.3	V	V	FB	FB	V	V	FB	V	V
1	V	V	FB	FB	V	V	FB	V	FB
3	FB	FB	FB	FB	FB	V	FB	V	FB
10	OF	FB	OF	OF	FB	FB	OF	V	FB
30	—	—	OF	—	—	—	—	—	—
100	—	—	OF	—	—	—	—	—	—

V = vegetative. FB = flower buds. OF = open flowers.

Bryophyllum crenatum is no rosette plant but internodes are much shorter under short-day than under long-day conditions. In all plants in which gibberellin application resulted in flower formation the uppermost nodes of the shoot showed marked elongation, exceeding that of plants grown in long days. Quite a similar growth pattern is also observed in plants which are induced to flower formation by transfer from long to short days (for some quantitative data, see ZEEVAART and LANG 1962).

Discussion

If the nine gibberellins are grouped, according to their activity in causing flower formation in the five plants tested, and considering those effective at the relatively lowest dosage level as highly active, those effective at the next higher levels as less active, *etc.*, the following picture is obtained:

Degree of activity	*Myosotis*	*Centaurium*	*Silene*	*Crepis*	*Bryophyllum*
Highest	A_7	A_3	A_7	A_4, A_7	A_3, A_4, A_7
Medium	A_1	A_1, A_4, A_5, A_7, A_9		A_1, A_3	A_1, A_2, A_5, A_9
Lowest		A_6		A_2, A_5, A_9	A_6
Inactive	A_2—A_6, A_8, A_9	A_2, A_8	A_1—A_6, A_8, A_9	A_6, A_8	A_8

The list reveals certain generalities. Thus, GA_7 appears almost always in the uppermost class, GA_8 always in the "inactive" class, A_1 usually in one of the two intermediate ones, *etc.* However, a closer inspection shows some clear differences in the response of different species. Thus, GA_7 was the most, or one of the most active gibberellins, with respect to flower induction, in *Myosotis*, *Silene*, *Crepis* and *Bryophyllum* but had a distinctly lower position in *Centaurium*. GA_4 was highly active in *Crepis* and *Bryophyllum* but distinctly less active in *Centaurium*, *Silene* and *Myosotis*. GA_1 was inferior to GA_3 in *Centaurium* but superior in *Myosotis*. GA_2 had little or no flower-inducing activity in most of the plants tested but was distinctly active in *Bryophyllum*.

The results permit two conclusions: (1) Gibberellins A_1 through A_9 differ considerably in their capacity to induce flower formation in cold-requiring, long-day and long-short-day plants grown under non-inductive temperature or light regimes; (2) the order of flower-inducing activity of these gibberellins is not the same in all plants[1].

This situation agrees with that found in other systems and described in the introduction[2]. A general conclusion that follows from this situation is that it is not legitimate to generalize results, concerning a given response, obtained with any single gibberellin in any single plant. This is particularly true if the results happen to be negative. With respect to flower formation, it appears quite likely that some of the negative results which have been reported in certain species were caused by the use of a gibberellin relatively ineffective in this response in the particular species. It may even be of interest to test the effect of some of the "new" gibberellins (particularly GA_7) on short-day plants, in which so far no flower formation has been obtained as a result of gibberellin treatment under strict long-day conditions. However, it would be premature to claim, on the basis of our results, that *all* negative results in flower induction with gibberellins can be explained in this manner. The fact remains that different species exhibit marked differences in their flowering response to gibberellin application; even if the responses to the most effective gibberellins are compared, *Silene* remains much less sensitive than *Crepis* — a difference which is *not* paralleled by an equally great difference in sensitivity to photoinduction.

[1] We had the privilege of seeing the results of similar experiments with lettuce *(Lactuca sativa)*, conducted by Drs. S. H. WITTWER and M. J. BUKOVAC, Michigan State University (East Lansing, Mich., U.S.A.) and presently in press (WITTWER and BUKOVAC 1962). In this plant, the order of activity was GA_3 and GA_1 (100 per cent plants flowering at a level of 0.09 μmoles); GA_7 (70 per cent); GA_4, GA_5 and GA_9 (30—40 per cent); GA_2 (10 per cent); GA_6 and GA_8 (none). The results point to quite the same conclusions as ours.

[2] The paper by WITTWER and BUKOVAC (1962) contains further illustrations of the specificity of the nine gibberellins in various growth responses.

It has been pointed out on previous occasions (see LANG 1957, 1961; LANG and REINHARD 1961) that in most — although not all — cases gibberellin-induced flower initiation is preceded by considerably more stem elongation than flower initiation induced by thermo- or photoinductive treatment. The results with gibberellins A_1 through A_9 generally confirm this relation. All plants of *Myosotis*, *Centaurium* and *Silene* which initiated flowers in response to gibberellin treatment exhibited a large degree of stem elongation; in *Bryophyllum*, they exhibited marked elongation of the uppermost stem internodes. A careful inspection of the data shows, however, that the correlation of stem elongation anf flower initiation in at least some of these plants is not absolute. Thus, GA_3 at levels of 30 μg and 100 μg per application caused either greater *(Myosotis)* or at least equal *(Silene)* stem elongation as GA_7 at 10 μg but did *not* result in flower formation. It thus cannot be claimed that flower formation occurs always, *quasi* automatically, once a certain amount of stem elongation has been reached. A significant corollary of this fact is that the effectiveness of different gibberellins on stem elongation and flower formation is *not* necessarily exactly the same.

In *Crepis* it appeared that any degree of stem elongation was associated with flower initiation, indicating that in this plant the two phenomena are very closely linked.

The main chemical differences between gibberellins A_1 through A_9 lie in the number and position of hydroxyl groups on the A and C rings, and the presence or absence of a double bond in the A ring (GROVE *et al.* 1961; MACMILLAN *et al.* 1961). The numbers of hydroxyl groups and double bonds vary as follows:

	GA_1	GA_2	GA_3	GA_4	GA_5	GA_6	GA_7	GA_8	GA_9
Hydroxyl groups . . .	2	1	2	1	1	2	1	3	0
Double bonds in A ring	0	0	1	0	1	0	1	0	0

There is no obvious correlation of elongation- and/or flower-inducing activity with these structural properties of the nine gibberellins. BRIAN and HEMMING (1961) pointed out that the three gibberellins which have high activity in cucurbits (GA_4, GA_7, GA_9) have one common property, namely absence of a hydroxyl in position 7 (C ring). With respect to flower-inducing activity, this rule does not seem to hold, for GA_7 in this respect held usually a high position, GA_4 a fairly high but GA_9 mostly a low one. At the present, it does not seem feasible to explain the difference in the activities of the different gibberellins exclusively in terms of their chemical structure[1].

[1] An obvious question is whether the different activities of the nine gibberellins are attributable, in whole or in part, to different penetration into the plant. This question needs experimental study. However, a number of observations and con-

It should also perhaps be pointed out that all gibberellins which proved to have the highest flower-inducing activity (GA_3, GA_4, GA_7) are gibberellins which so far have been unequivocally proven to exist only in the mold *Fusarium*. It thus certainly cannot be stated at present that gibberellins of higher-plant origin were more active in responses specific to higher plants than the fungal gibberellins. However, it seems possible that GA_3 and GA_7 do occur in higher plants, namely germinating barley (apparently in a bound form) and the endosperm of *Echinocystis macrocarpa*, a cucurbit (LAZER *et al.* 1961 and WEST and REILLY 1961, resp.). The latter case would be of particular interest since GA_7 is one of the gibberellins especially active on growth in cucurbits and since preparations from *Echinocystis* endosperm have been found to possess very marked flower-inducing activity (LANG *at al.* 1957).

Summary

The effect of gibberellins A_1 through A_9 on stem elongation and flower formation in five plants was tested. The plants were *Myosotis alpestris* and a biennial strain of *Centaurium minus* (cold-requiring plants), *Silene armeria* and *Crepis parviflora* (long-day plants), and *Bryophyllum crenatum* (a long-short-day plant). The two former plants were maintained on non-inductive temperatures and long days, the three latter on short days, In *Myosotis*, flower formation was only obtained with GA_7 and GA_1, the latter being relatively less active. In *Centaurium*, GA_3 was the most effective, followed by GA_1, GA_4 and GA_7 and perhaps GA_5 and GA_9. In *Silene*, flower formations was induced only by GA_7. In *Crepis*, the most effective gibberellins were GA_4 and GA_7, in *Bryophyllum*, GA_3, GA_4 and GA_7. Thus, the different gibberellins exhibited considerable differences in their activity with respect to flower induction, and different plants exhibited in this respect certain specific differences in their sensitivity to the various gibberellins. Except in *Crepis*, flower initiation as a result of gibberellin treatment was always preceded by substantial stem or internode elongation; however, the correlation between the effect of the different gibberellins on stem elongation and flower induction was not in all cases complete. No correlation of the flower-inducing and elongation-promoting activity with the chemical structure of the different gibberellins could be recognized.

siderations do not render this a very likely possibility. Firstly, the differences in chemical structure between most of the gibberellins, including active and less active ones, are not suggestive of large differences in penetrability. Secondly, the differences in the order of activity which are found in different plants are likewise not indicative of general differences in penetration. Lastly, the fact that the order of activity of the gibberellins in two different responses of *one and the same* plant — like in flower formation and stem elongation in our own experiments, or germination and hypocotyl elongation as described by BRIAN *et al.* (1962) — can be different cannot be readily explained with differences in penetration.

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