REACTION OF *HELENIUM AMARUM* TO GIBBERELLIC ACID

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ABSTRACT

Gross responses of the species *Helenium amarum* (Raf.) H. Rock to various dosages of gibberellin, as a substitute for long photoperoids, were studied. Gibberellin A ± X ($GA_1 + GA_3$) and potassium salt of gibberellic acid (KGA_3, 75% acid) were used. The calculation of the concentration of KGA_3 was based upon the acid part of KGA_3 only. Results are summarized as follows:

1. The elevation of rosette leaves required a minimum of 0.0025 microgram/plant of $GA_1 + GA_3$ or KGA_3.
2. The elongation of rosette leaves required a minimum of 0.0025 microgram/plant of $GA_1 + GA_3$ or KGA_3.
3. Initiation of bolting required a minimum of 0.511 microgram/plant grown on soil ($GA_1 + GA_3$) or 0.412 microgram/plant (KGA_3).
4. Initiation of flower formation required a minimum of 1.482 microgram/plant of $GA_1 + GA_3$ or 1.450 microgram/plant of KGA_3.

It was concluded that the elevation and elongation of rosette leaves may serve as criteria for the bioassay of gibberellin. At the 5% level of significance, responses were significant with each 10-fold increment of gibberellin concentration. The Log dose/Log response curves are linear in the range from 0.0025 to 0.25 microgram/plant.

INTRODUCTION

The series of experiments reported here had two purposes. The first was to evaluate carefully the gross responses of the species *Helenium amarum* (Raf.) H. Rock, bitterweed, to various dosages of gibberellins as a substitute for long photoperiods. The second was to determine the validity and possible advantages of utilizing the rosettes of bitterweed as organisms for the bioassay of gibberellins.

Helenium amarum is a common roadside weed and pasture pest of the southeastern United States. Its present range extends from southern Texas, throughout the Gulf Coastal Plain and into Cuba. It is commonly found as far northward as southern Illinois, Indiana, and Central Missouri. It has been reported from Massachusetts, and recently from California.

Many of the autecological aspects of bitterweed have been studied by Caplenor and associates (1960, 1961 and unpublished data). They have shown that it normally exists as a winter annual, at least in the Gulf Coastal Plain area. There, seeds germinate in the autumn. Young plants overwinter as rosettes, and bolt in April and early May. Subsequent observations indicate that toward the northern limit of its range many of the seeds do not germinate until late winter or spring, the plant assuming a typical annual life cycle.

The life cycle and growth form of the species indicates that it is a long day plant with diameter of rosette, number of leaves, time of bolting and time of flowering directly related to length of the photoperiod. It has been shown that the potassium salt of gibberellic acid can substitute for the long photoperiod in the induction of bolting. Preliminary experiments did not attempt to discover a quantitative response, nor did they include a study of the relationship between dosages of gibberellins and flowering. Observations did indicate rapid responses by rosettes grown on short photoperiods to both long photoperiods and gibberellins. These responses were (1) elevation of the rosette leaves and (2) elongation of them.

Nine gibberellins have been structurally identified: gibberellin A_1, gibberellin A_2, gibberellin A_3, gibberellin A_4, gibberellin A_5, gibberellin A_6, gibberellin A_7, gibberellin A_8, gibberellin A_9. The common gibberellic acid is gibberellin A_3. GA_1, GA_2, GA_3, GA_4, GA_7, GA_9 were isolated from the fungus *Gibberella fujikuroi* (Saw.) Wr. (Grove et al. 1960, Brian 1961 Cross et al. 1962). GA_1, GA_5, GA_6, GA_8, were isolated from immature seed of runner bean *Phaseolus multiflorus* Lam. (MacMillan and Suter 1958; MacMillan et al. 1959; MacMillan 1960, 1962). GA_1, and GA_5, were found in *Phaseolus vulgaris* L. These two gibberellins were once called Bean factor I and Bean factor II respectively (MacMillan et al. 1959, West and Phinney 1959, West 1961). GA_1 was isolated from water sprouts of *Citrus unshiu* Marcov. (Sumiki and Kawarada 1961). Elson et al (1964) reported that GA_1, GA_3, GA_4, GA_7, and some other unknown compounds were isolated from an acidic extract of the seed *Echinocystis macrocarpa* Greene, the first time GA_4, and GA_7, had been identified in the extract of a flowering plant. Besides these known gibberellins, there are gibberellin-like substances which have been extracted from members of the plant kingdom (fungi, mosses, monocotyledonae, dicotyledonae) Radley 1958, Phinney and West 1960, Adler et al. 1961, Kato et al. 1962, Kato 1963).

Gibberellins affect many physiological responses of plants. They increase the number and length of cells, promote vegetative growth, terminate dormacy, and induce flowering and parthenocarpy (Brian 1961, Phinney and West 1961, Stuart and Cathey 1961). The effectiveness of gibberellins in biological activity varies with structural configuration, concentration, and with the plant or organ of the plant used for bioassay (Halevy and Cathey 1960, Brian et al. 1962, Wittwer and Bukovac 1962). The technique of gibberellin extraction is based upon a chromotagraphic technique (Phinney et al. 1957, Bentley 1962, MacMillan et al. 1962). The qualitative and/or quantitive assay of gibberellin is based on physico-chemical properties or on physiological properties. Infrared spectrophotometry, fluorimetry, polarography and isotopic labeling methods have been used (Phineey and West 1961). Criteria for biological assays include elongation of dwarf pea stem (Brian and Hemming 1955, Brian et al. 1958, McComb and Carr 1958), elongation of leaf section of wheat seedling (Skene and Carr 1961), elongation of first oat leaf (Michniewicz 1961), elongation of leaf sheath of dwarf *Zea mays* (Neely and Phinney 1957), (Phinney 1961), elongation of cucumber tendril (Galun 1959), elongation of cucumber hypocotyl (Brian and Hemming 1961), germination of excized embryos of *Avena*

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fatua L. (Naylor and Simpson 1961), angle between lamina and sheath of intact rice seedlings (Maeda 1962). Maeda (1962) may have been the first one who introduced angular growth to determine the response to gibberellin treament. He found that gibberellin treatment increased the extension of the adaxial side of lamina, therefore increased the angle between the sheath and lamina. The response was qualitative.

Materials and Methods

Seeds of *Helenium amarum* were collected on September 22, 1963, in Smith County, Tennessee. They were germinated in Hoagland and Arnon's nutrient solution number 1 (Hoagland and Arnon 1950) and transplanted onto pots or culture dishes, one plant per dish or pot.

All plants were grown in the Peabody College greenhouse from January 8, 1964, until termination of the experiments (June 3, 1964). They were kept at a 10 hour photoperiod (8 A.M. to 6 P.M.) by hand manipulating a black plastic cover.

A soil for growth of plants was prepared by mixing topsoil thoroughly with peat moss (approximately 2:1). Agar cultures were made by adding 15 grams of agar to one liter of the nutrient solution.

Nutrient solution was given at two-day intervals to the soil cultures and at ten-day intervals to the agar cultures, and tap water was given daily. Temperature was recorded as the mean of minimum and the mean of maximum daily temperatures (minimum: 70. 76 ± 0. 78° F, maximum: 105.70 ± 1.96° F). Natural light was used. The greenhouse was sprayed with lime solution on April 24, 1964 to lower the intensity of light. The humidity was not controlled.

The stock solution of gibberellin was made by dissolving weighed gibberellin into weighed warm distilled water. It was stored in a refrigerator at aproximately 4 degrees centigrade. The stock solution was used within 30 days after preparation. Different concentrations of gibberellin solution were made at the time of treatment and were discarded after treatment. The plants were treated by dropping 5 microliters of gibberellin solution on the shot apex from a Hamilton microliter syringe #701-NCH.

Two series of experiments were performed: the first one with gibberellin A + X ($GA_1 + GA_3$) from Mann Research Laboratories, Inc., New York, New York; the second one with gibberellic acid (Potassium salt, 75% acid) (KGA_3) from Nutritional Biochemicals Corporation, Cleveland, Ohio.

Series 1

A solution of $GA_1 + GA_3$ was made by dissolving 0.100 grams of $GA_1 + GA_3$ in 200 grams of warm distilled water.

Plants were separated into 12 groups of ten plants per group. There were six groups growing in soil: six in agar. Amounts of gibberellin applied to each plant in each group were 0.000005 (1), 0.00025 (2), 0.0025 (3), 0.025 (4) and 0.25 (5) micrograms. Group O was the control group.

Plants were treated on March 2, 1964. The results were recorded as follows:

1. The magnitude of angles between marked leaves and the horizontal surface of the substrate. These angles were determined by measuring the distance from the substrate to the leaves, at a point on the substrate 2 cm from the stem. The three most prostrate leaves were marked on each plant 24 hours before treatment to insure uniformity.
2. After four days of treatment the three largest angles formed by any three leaves on each plant (at least 25 mm long) with the surface of the substrate were measured, using a protractor.
3. After angular response had been determined, a known amount of $GA_1 + GA_3$ was applied to the plants at seven-day intervals to attempt to induce bolting and flowering. The dates of bolting and flowering were recorded, as were the amounts of gibberellin required to induce each.

Series 2

In this series, we used KGA_3 (75% acid) on one experimental group, and $GA_1 + GA_3$ on another. Since the elongation of leaves of treated plants was obvious in the first series, leaf elongation and angular response were both measured in the second series.

A stock solution of $GA_1 + GA_3$ was prepared as described in the first series. Its concentration was 500 ppm of gibberellin. A stock solution of potassium salt of gibberellic acid (75% acid) was prepared by dissolving 0.100 gram of KGA_3 in 250 grams of warm distilled water. The calculations of the KGA_3 concentration in solution were based upon the acid part of KGA_3 only. Therefore the concentration of stock solution was 300 ppm of gibberellic acid. Amounts of gibberellin applied to plants growing in soil were 0.0025 (1), 0.025 (2), 0.05 (3), 0.10 (4), 0.2 (5) micrograms. Group O was held as the control group.

Figure 1. Control plant.

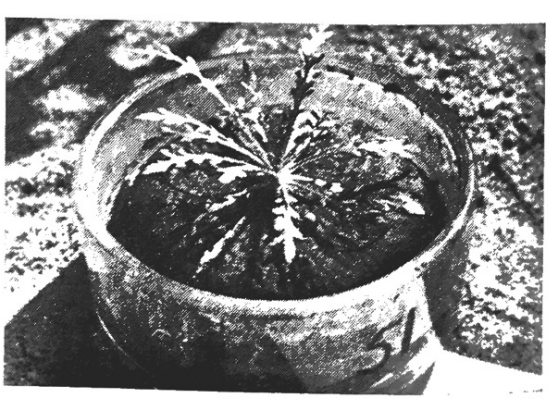

Figure 2. Plant from group 5, 0.25 microgram gibberellin per plant.

Plants were treated on April 21, 1964. Responses were measured as follows:

1. For each plant, the largest angles formed by the elevation of leaves exceeding 25 mm in length were measured on April 26, 1964, using a protractor. The surface of the substrate served as a base for the angular measurement.
2. The elongation of marked leaves (1 leaf per plant) was measured on April 24, 1964. Young leaves (6 to 8 mm long) were chosen 24 hours before treatment.
3. After determining the angular and elongation responses, the $GA_1 + GA_3$-treated plants were abandoned and the KGA_3-treated plants were kept on treatment. A known amount of KGA_3 was applied to the later group at seven-day intervals to induce bolting and flowering. The dates of bolting and/or flowering were recorded, along with the amounts of KGA_3 required to produce the effects.

In both series, data on elongation and angular response were analyzed statistically by use of the "t" test. The amount of gibberellin needed for the induction of bolting and the induction of flower formation were stated as the arithmetic mean of the individual data.

Results

Series 1

For the general view of the reaction of bitterweed rosettes to gibberellin see Figures 1 and 2. The results of uplift of leaf in mm. as measured from lowest leaves are show nin Table I. The results of the response as measured by three largest angles are presented in Table II. The results of the induction of bolting and flowering are recorded in Table III.

In soil culture, the rosettes reacted positively to as little as 0.000005 microgram per plant of $GA_1 + GA_3$ by uplifting rosette leaves. Yet, irregularity of results are such that it is only possible to state with assurance that as little as 0.0025 micrograms of $GA_1 + GA_3$ can be detected by this effect. Beyond that amount, tenfold increments of the gibberellin do not result in stepwise detection of concentrations by this method. Plants growing in agar were apparently less sensitive than those in soil.

Table I

Effect of Treatment with $GA_1 + GA_3$ upon the Distance 'd'* (Uplift of Leaf in Millimeters).

Group	No. of observations		Mean ± Standard error of the mean	
	Agar	Soil	Agar	Soil
0	25	29	2.18 ± 0.42	0.86 ± 0.20
1	27	30	2.46 ± 0.45	1.58 + 0.24
2	22	30	3.27 ± 0.51	1.41 ± 0.38
3	27	30	3.65 ± 0.54	1.93 ± 0.40
4	29	30	2.86 ± 0.51	1.50 ± 0.24
5	30	30	3.67 ± 0.56	2.58 ± 0.38

* 'd' is distance from the substrate to the leaves, at a point on the substrate 2 cm from the stem.

Table II

Effect of Treatment with $GA_1 \pm GA_3$ upon Angular Response in Degrees.

Group	No. of observations		Mean + Standard error of the mean	
	Agar	Soil	Agar	Soil
0	30	30	12.23 ± 2.46	4.90 ± 1.53
1	30	30	13.30 ± 2.85	7.43 ± 1.07
2	22	30	10.59 ± 2.90	7.79 ± 1.70
3	24	30	32.71 ± 3.22	22.37 ± 2.97
4	30	30	53.60 ± 1.97	34.13 ± 1.84
5	30	30	64.20 ± 1.40	51.83 ± 1.84

Table III

Amounts of $GA_1 + GA_3$ (Microgram Per Plant) Needed to Induce Bolting and/or Flowering

Condition		Experimental group				Control group			
						No treatment		No treatment beyond bolting	
		Number of plants treated only to bolting	Number of plants treated beyond bolting	Number of plants bolting or flowering	Mean + Standard error of the mean (microgram of gibberellin per plant)	Number of plants	Number of plants bolting or flowering	Number of plants	Number of plants flowering
Plants on agar	B*	50		48	0.511 + 0.034	10	0		
	F**	50	34	0		10	0	14	0
Plants on soil	B*	50		50	0.552 ± 0.028	10	1		
	F**	50	30	20	1.482 ± 0.040	10	0	20	0

*Bolting.
**Flowering.

When using the angular response of young leaves as a criterion for detection, 0.0025 microgram was again the smallest quantity of $GA_1 + GA_3$ giving consistently positive results. However, all tenfold increments above that quantity were quantitatively detectable. In this experiment, data taken from plants growing on agar and those taken from plants on soil were similar. Angular responses, as demonstrated in this experiment, are depicted graphically in Figure 3. This indicates linear response to the various concentrations.

Series 2

The results of the angular response by the measurement of three largest angles are recorded in Table IV; the results of the elongation response in Table V. The results of the induction of bolting and flowering are recorded in Table VI.

TABLE IV

THE ANGULAR RESPONSE TO TREATMENT WITH KGA_3 (GROUPS 1, 2, 3, 4, AND 5) AND $GA_1 + GA_3$ (GROUPS X2, X3, X4 AND X5). GROUP 0 IS A CONTROL GROUP. THE RESPONSE WAS RECORDED IN DEGREES.

Group	Number of observations	Mean + Standard error of the mean
0	30	30.57 ± 2.64
1	30	41.50 + 2.45
2	30	43.03 ± 2.46
3	30	53.87 + 2.26
4	30	64.37 ± 2.59
5	30	70.47 + 1.84
X2	9	40.33 ± 3.02
X3	9	42.33 + 3.23
X4	9	69.22 ± 2.42
X5	9	68.89 + 2.83

TABLE V

THE ELONGATION OF LEAVES IN RESPONSE TO TREATMENT WITH KGA_3 (GROUPS 1, 2, 3, 4, AND 5); TO TREATMENT WITH $GA_1 + GA_3$ (GROUPS X2, X3, X4, AND X5). (DISTANCES RECORDED IN MILLIMETERS.)

Group	Number of observations	Mean + Standard error of the mean
0	10	27.20 ± 1.97
1	10	34.70 ± 1.16
2	10	39.45 ± 1.54
3	10	37.20 ± 0.77
4	10	39.80 ± 1.53
5	10	43.80 ± 1.32
X2	3	40.66 ± 2.34
X3	3	38.00 ± 1.16
X4	3	37.66 ± 3.84
X5	3	38.66 ± 5.33

As in the experiments in Series 1, 0.0025 micrograms of gibberellic acid was clearly detectable. In this experiment the difference between responses to 0.0025 micrograms and 0.02 micrograms of gibberellic acid was not significant. Other increments, excepting that from 0.10 mg to 0.25 mg, gave significantly different responses. These responses are depicted graphically in Figure 4.

Using leaf elongation as a quantitative criterion, the smallest detectable quantity of gibberellic acid was 0.0025 microgram per plant. Responses to increments of KGA_3 were consistently significant with tenfold increments only, responses of group 3 and group 4 (two-fold increments) were not significant. They are depicted graphically in Figure 5.

TABLE VI

THE AMOUNTS OF KGA_3 (MICROGRAMS PER PLANT) NEEDED TO INDUCE BOLTING AND/OR FLOWERING

Condition	Experimental Group				Control group			
					No treatment		No treatment beyond bolting	
	Number of plants treated only to bolting	Number of plants treated belyond bolting	Number of plants bolting or flowering	Mean + Standard error of the mean (micrograms of gibberellin per plant)	Number of plants	Number of plants bolting or flowering	Number of plants	Number of plants flowering
Bolting	50		50	0.412 ± 0.015	10	1		
Flowering	50	26	3	1.450 ± 0.052	10	0	24	0

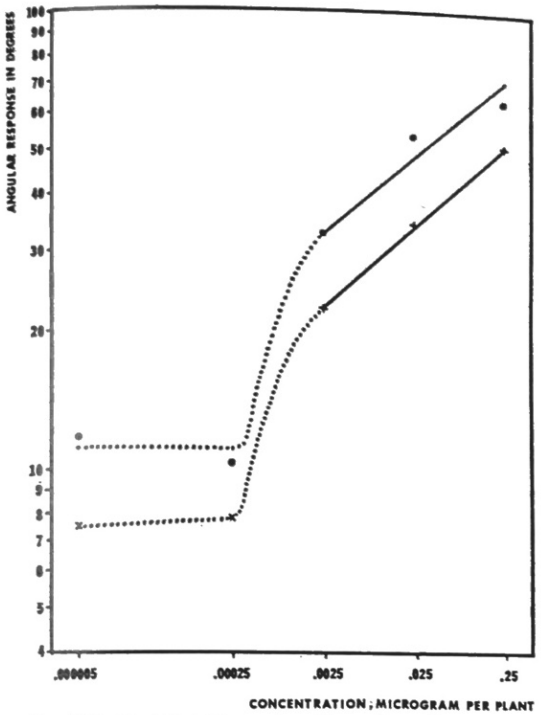

Figure 3. Dosage response curve (log dose, log response) for $GA_1 + GA_3$ using the elevation of rosette leaves for bioassay. For the plants growing on soil, each point represents 30 measurements; for the plants growing on agar 22 to 30 measurements.

• plants growing on agar.
x plants growing on soil.
— significant.
.... non-significant.

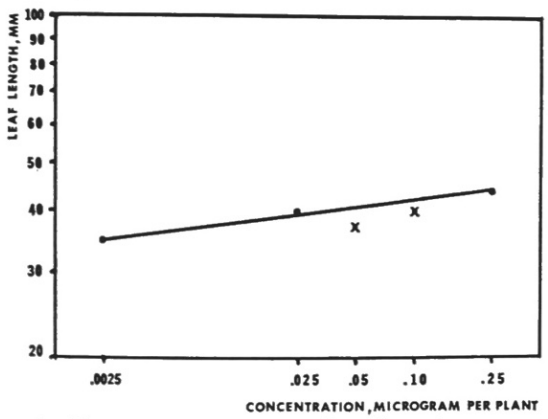

Figure 4. Dosage response curve (log dose, log response) for KGA_3 using the elevation of rosette leaves for bioassay. Each point represents 30 measurements.

• significant point.
x non-significant point.

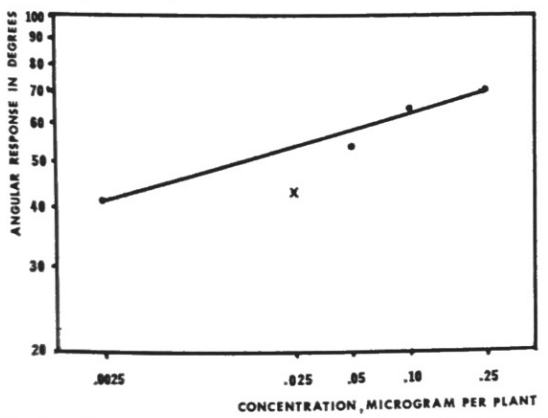

Figure 5. Dosage response curve (log dose, log response) for KGA_3 using the elongation of the rosette leaves for bioassay. Each point represents 10 measurements.

• significant point.
x non-significant point.

The data indicate no difference in response to $GA_1 + GA_3$ and KGA_3 in equal amounts.

Discussion

Results indicate a number of definite reactions to application of gibberellins by bitterweed held on short photoperiod. These responses are, in chronological order of their occurrence, (1) elevation of the apices of rosette leaves, (2) elongation of rosette leaves, (3) bolting, and (4) blooming. Under conditions of these experiments, mean total minimal amounts of gibberellin ($GA_1 + GA_3$ or KGA_3) required to elicit these responses were, respectively, (1) 0.0025 microgram per plant, (2) 0.0025 microgram per plant, (3) 0.511 microgram per plant ($GA_1 + GA_3$, plants growing on agar), 0.552 microgram per plant ($GA_1 + GA_3$, plants growing on soil), or 0.412 microgram per plant (KGA_3), and (4) 1.482 microgram per plant ($GA_1 + GA_3$), or 1.450 microgram per plant (KGA_3). Since these responses, with the possible exception of leaf elongation, are also natural responses to long photoperiod, it appears that the gibberellins substitute for some natural product formed only in response to long (12 hour) photoperiods. The responses to long photoperiod and to gibberellins are also similar in that they do not truly induce the plants to flower. Both long photoperiods and treatment with gibberellins must be continued beyond an initial phase or blooming will not occur. Another difference in effect of treatment which may be quantitative rather than qualitative is that very young seedlings react more positively to treatment with gibberellins than to long photoperiods (Caplenor, unpublished data). Chailakhian (1961) has reported that a greater quantity of gibberellin-like substances can be extracted from long-day plants kept on long photoperiods than when they are kept on short photoperiods. This work suggested that in long-day plants the level of endogenous gibberellins or gibberellin-like substances varies proportionally with the photoperiods. It explains why seedlings too immature to bolt on very long photoperiods can be induced to do so precociously by treatment with gibberellins; because seedlings are able to react to gibberellin-like substances before they can be photoperiodically induced to produce them. Thus the response mechanism is apparently developed before the synthetic mechanism.

No literature was found relating the elevation of leaves of rosette plants in response to treatment with gibberellins. Maeda (1962) reported the movement of the lamina toward the abaxial side after treating rice seedlings with gibberellins. His previous work had shown that the lamina joint participates in the bending movement of laminae toward their abaxial side and that there was an increment of cell length at the abaxial side. In bitterweed experiments the leaves were elevated toward their abaxial side. This suggests either an increase in cell number and/or cell length on the abaxial side of the leaf or some osmotic disturbance of cells resulting in increased turgor pressure on the abaxial side.

In the first work on bitterweed, the responses of basal leaves were chosen because of ease of measurement.

Later experiments showed that leaves near the apex were more responsive. The basal leaves are oldest and are least reactive. This is probably because the site of gibberellin activity is at the meristematic region of the apex (Bradley and Crane 1957, Sachs et al. 1959, Koller et al. 1960, Evtushenko 1961, Sachs and Lang 1961). Both methods of measurement of the elevation of leaves after treatment with gibberellins indicated that responses were more regular and significant in young leaves than in basal leaves.

Gibberellin has been shown to cause bolting and/or flowering of plants grown under non-inductive conditions (Lang and Reinhard 1961). Bolting was a consistent response (Lang 1956a, 1957), but induction of floral formation by gibberellins varied with physiological types of plants (longday, shortday, cold-requiring) and with the conditions under which the plants were grown (Lang 1956b, Doorenbos and Wellensiek 1959, Dostal 1959, Bukovac and Davidson 1959, Harada and Nitsch 1959). Gibberellins induce the flowering of longday plants grown in shortday conditions, but they will not substitute for short photoperiod in shortday plants or for cold in cold requiring plants. This fact leads to many attempts to explain the flowering mechanism. Lang (1957) suggested that gibberellin probably functions in conjunction with inhibitory factors and that a response is the display of promotive processes versus inhibitory processes. Lincoln and Hammer (1958) suggested that gibberellin promotes the capacity for the storage of the floral stimulus in the immature tissue. Chailakhian (1961) suggested that floral formation is controlled by flowering hormones (florigen) composed of two factors: gibberellin necessary for vegetative growth and anthesin necessary for flowering. Most published results indicate that there are at least two factors involved in the initiation of flowering. If this is the case, the terminal factor (anthesin) has not been isolated and identified. Our work suggests that, although gibberellins added as a single dose are not inducive, a specific amount of gibberellins applied over a period of time constitutes the only external requirement for the initiation of flowering of bitterweed.

Since preliminary observations had indicated an almost immediate response by bitterweed rosettes to treatment with gibberellins, one of the purposes of this series of experiments was to test their adaptability as organisms for the bioassay of gibberellins. If acceptable they should have certain advantages over some organisms in current usage. These advantages are that (1) they are easily and quickly grown without specialized facilities, (2) the response is definite and rapid, and (3) the responses are easily measured with common rulers and/or protractors. Evident responses which are rapid enough to have possible use in bioassay are the elevation and elongation of rosette leaves. Both of these responses were tested. Results are shown in Tables II, IV, and V and in Figures 1, 2, and 3. Table VII relates the results of these attempts to those of other experimenters.

TABLE VII

SUMMARY OF DIFFERENT METHODS OF BIOASSAY FOR GIBBERELLIN

Organism	Response	Method of Treatment	Sensitivity	Type of Curve
Dwarf pea stem (McComb & Carr 1958)	Percentage of stem elongation	4 microliter drop in the axil of third node	0.0001 to 5 micrograms per plant	Linear Log dose/Log response
Dwarf *Zea Mays* (Phinney 1961)	Elongation of the first leaf sheath	0.1 ml test solution into first unfolding leaf	0.001 to 1.0 microgram per plant	Linear Log response/Log dose
Wheat leaf section (Skene & Carr 1961)	Elongation of leaf sections	10 segments in 5 ml of test solution	10^{-3} ppm to 10^{-1} ppm solution per incubation	Non-linear Response/Log dose
Oat leaf (Michniewicz 1961)	Elongation of the first leaf section	12 sections in 0.5 to 1 ml test solution	0.001 to 10 micrograms per ml of solution	Non-linear Response/Log dose
Cucumber tendril (Galun 1959)	Elongation	15 sections in test solution	0.33 ppm to 3.3 ppm solution per incubation	Non-linear Response/Log dose
Excised embryo of *Avena sativa* (Naylor & Simpson 1961)	Percentage of germination	25 embryos in 0.25 ml of test solution	10^{-3} ppm to 10^{-1} ppm solution per incubation	Linear Response/Log dose
Bitterweed rosette	Leaf elevation or leaf elongation	5 microliter drop on the apex	0.0025 to 0.25 micrograms per plant	Linear Log response/Log dose

In detection of minimal amounts of gibberellin, gross responses of bitterweed are less sensitive than the dwarf pea method and the method involving germination of *Avena sativa* excised embryos. It is the same order (thousandths of micrograms) as other methods. Leaf elongation and angular leaf elevation are clearly linear responses (Log response/Log dose). Such response leads to obvious advantages in interpolation. The obvious disadvantage of the bitterweed method is that it could not be used directly to test chromatograms of extracts as could the germination and leaf section methods.

It seems clear that gross responses of bitterweed rosettes may serve for the bioassay of gibberellins. Even though the tests are not as sensitive as some previously devised, there are certain real advantages in the methods described here. The response is rapid and linear: sensitivity is in the order of thousandths of micrograms, and no specialized growth or measuring devices are required.

Literature Cited

Adler, N., T. Medwick, R. G. Johl, and M. A. Manzelli. 1961. Evidence for the widespread occurrence of a gibberellic acid-like substance in higher plants. Advances in Chemistry Series No. 28: 26-36.

Bentley, J. A. 1962. Analysis of plant hormones, pp. 75-125. In D. Glick (ed.), Methods of biochemical analysis. Vol. IX. J. Wiley and Sons, Inc., New York and London.

Bradley, M. V., and J. C. Crane. 1957. Gibberellin-stimulated cambial activity in stems of apricot spur shoots. Science 126: 972-973.

Brian, P. W. 1961. The Gibberellins: A new group of plant hormones. Science progress 49: 1-16.

Brian, P. W., and H. G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. Physiol. Planta. 8: 669-681.

———. 1961. Promotion of cucumber hypocotyl growth by two new gibberellins. Nature 189: 74.

Brian, P. W., H. G. Hemming, and D. Lowe. 1958. Effect of gibberellic acid on rate of extension and maturation of pea internodes. Ann. Bot. London (New Series) 22: 593-542.

———. 1962. Relative activity of the gibberellins. Nature 193: 946-948.

Bukovac, M. J., and H. Davidson. 1959. Gibberellin effects on photoperiod-controlled growth of *Weigelia*. Nature 183: 59-60.

Caplenor, D. 1960. Unpublished data.

———. 1960. A preliminary report on the autecology of *Helenium amarum* (Compositae). (Abstract) Jour. Tenn. Acad. Sci. 35: 103.

———. 1961. The life cycle of Helenium amarus. Ecol. 42: 843-844.

Chailakhian, M. Kh. 1961. Effect of gibberellins and derivatives of nucleic acid metabolism on plant growth and flowering, pp. 531-542. In R. M. Klein (ed.), Plant Growth Regulation. Iowa State Univ. Press, Iowa.

Cross, B. E., R. H. B. Galt, and J. R. Hanson. 1962. New metabolites of *Gibberella fujikuroi* I. Gibberellin A_7, Gibberellin A_9. Tetrahedron 18: 451-459.

Doorenbos, J., and S. J. Wellensiek. 1950. Photoperiodic control of floral induction. Ann. Rev. Plant Physiol. 10: 147-184.

Dostal, R. 1959. Gibberellic acid and growth correlations. Nature 183: 1338.

Elson, G. W., D. F. Jones, J. MacMillan, and P. J. Suter. 1964. Plant hormones IV: Identification of the gibberellins of *Echinocystis macrocarpa* Greene (Cucurbitaceae) by thin layer chromatography. Phytochemistry 3: 93-101.

Evtushenko, G. A. 1961. Localization of the gibberellin reaction in plants. Translated from Fiziol Rasteni 8: 304-308.

Galun, E. 1959. The cucumber tendril—a new test organ for gibberellic acid. Experientia 15: 184-185.

Grove, J. F., J. MacMillan, T. P. C. Mulholland, and W. B. Turner. 1960. Gibberellic acid. Part XVII: The stereochemistry of gibberic and epigibberic acid. Jour. the Chem. Soc. (London) 3049-3057.

Halvey, A. H., and H. M. Cathey. 1960. Effect of structure and concentration of gibberellins on the growth of cucumber seedlings. Bot. Gaz. 122: 63-67.

Harada, H., and J. P. Nitsch. 1959. Flower induction in Japanese chrysanthemums with gibberellic acid. Science 129: 777-778.

Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soils. Calif. Agr. Expt. Sta. Circ. 347.

Kato, Jiro. 1963. A gibberellin-like substance in bambo shots. Naturwissenschaften 50: 226.

Kato, J., W. K. Purves, and B. O. Phinney. 1962. Gibberellin-like substances in plants. Nature 196: 687-688.

Koller, D., H. R. Highkin, and O. H. Caso. 1960. Effects of gibberellic acid on stem apices of vernalizable grasses. Amer. Jour. Bot. 47: 518-524.

Lang, A. 1956a. Stem elongation in a rosette plant, induced by gibberellic acid. Naturwissenschaften 43: 257-258.

———. 1956b. Induction of flower formation in biennial *Hyoscyamus* by treatment with gibberellin. Naturwissenschaften 43: 284-285.

———. 1957. The effect of gibberellin upon flower formation. Proc. Nat. Acad. Sce. (Wash.) 43: 709-717.

Lang, A., and E. Reinhard. 1961. Gibberellins and flower formation. Adv. in Chem. Series No. 28: 71-79.

Lincoln, R. G., and K. C. Hamner. 1958. An effect of gibberellic acid on the flowering of *Xanthium*, a short day plant. Plant Physiol. 33: 101-104.

McComb, A. J., and D. J. Carr. 1958. Evidence from a dwarf pea bioassay for naturally occurring gibberellins in ght growing plant. Nature 181: 1548-1549.

MacMillan, J., and P. J. Suter. 1958. The occurrence of gibberellin A_1 in higher plants: Isolation from the seed of runner bean (*Phaseolus multi florus*). Naturwissenschaften 45: 46-47.

MacMillan, J., J. C. Seaton, and P. J. Suter. 1959. A new plant growth promotion acid—gibberellin A_5 from the seed of *Phaseolus multiflorus*. Proc. Chem. Soc. (London), p. 325.

———. 1960. Plant hormones. I. Isolation of gibberellin A_1 and gibberellin A_5 from *Phaseolus multiflorus*. Tetrahedron 11:60-66.

———. 1962. Plant hormones. II. Isolation and structures of gibberellin A_6 and gibberellin A_8. Tetrahedron 18: 349-335.

Maeda, Eizo. 1962. Studies on the mechanism of leaf formation in crop plants. III. Effects of gibberellin on the extension of lamina joints in intact rice seedlings. Proc. Crop Sci. Soc. (Japan) 31: 49-54.

Michniewicz, M. 1961. A biological assay for gibberellic acid with the first oat leaf. Acta Soc. Bot. Polon. 30: 553-568.

Naylor, J. M., and G. M. Simpson. 1961. Bioassay of gibberellic acid using excised embryo of *Avena fatua* L. Nature 192: 679-680.

Neely, P. M., and B. O. Phinney. 1957. The use of the mutant dwarf-1 of maize as a quantitative bioassay for gibberellin activity. Plant Physiol. (Suppl.) 32: xxxi.

Phinney, B. O. 1961. Dwarfing genes in *Zea mays* and their relation to the gibberellins, pp. 489-501. In R. M. Klein (ed.), Plant Growth Regulation. Iowa State Univ. Press, Iowa.

Phinney, B. O., and C. A. West. 1960. Gibberellins as native plant growth regulators. Ann. Rev. Plant Physiol. 11: 411-436.

———. 1961. Gibberellins and plant growth, pp. 1185-1227. In W. Ruhland (ed.), Encyclopedia of plant physiology, Vol. XIV, Springer-Verlag, Berlin-Gottingen-Heidelberg.

Phinney, B. O., C. A. West, M. Ritzel, and P. M. Neely. 1957. Evidence for "gibberellin-like" substances from flowering plants. Proc. Nat. Acad. Sci. (Wash.) 43: 398-404.

Radley, M. 1958. The Distribution of substances similar to gibberellic acid in higher plants. Ann. Bot. Lond. (New Series) 22: 297-307.

Sachs, R. M., and A. Lang. 1961. Shoot histogenesis and the subapical meristem: the action of gibberellic acid, Amo-1618, and Maleic hydrazide, pp. 567-578. In R. M. Klein (ed.), Plant Growth Regulation. Iowa State Univ Press, Iowa.

Sachs, R. C., C. F. Bretz, and A. Lang. 1959. Cell division and gibberellic acid. Exp. Cell. Res. 18: 230-244.

Skene, K. G. M., and D. J. Carr. 1961. Studies on a wheat leaf assay for the quantitative determination of gibberellin activity in plant extracts. Phyton Rev. Internac. Bot. Exptl. Argentina 16: 97-115.

Stuart, N. W., and H. M. Cathey. 1961. Applied aspects of the gibberellins. Ann. Rev. Plant Physiol. 12: 369-394.

Sumiki, Y., and A. Kawarada. 1961. Occurrence of gibberellin A_1 in the water sprouts of *Citrus*, pp. 483-487. In R. M. Klein (ed.), Plant Growth Regulation. Iowa State Univ. Press, Iowa.

West, C. A. 1961. The chemistry of gibberellins from flowering plants, pp. 473-482. In R. M. Klein (ed.), Plant Growth Regulation. Iowa State Univ. Press, Iowa.

West, C. A., and B. O. Phinney. 1959. Gibberellins from flowering plants. I. Isolation and properties of a gibberellin from *Phaseolus vulgaris* L. Jour. Amer. Chem. Soc. 81: 2424-2427.

Wittwer, S. H., and M. J. Bukovac. 1962. Quantitative and qualitative differences in plant response to the gibberellins. Amer. Jour. Bot. 49: 524-529.