EFFECTS OF GIBBERELLIC ACID ON THE GROWTH AND ALKALOIDAL CONTENT OF *DATURA STRAMONIUM* L.

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Gibberellic acid, at three dose levels, was injected into growing plants of *Datura stramonium* and the effects on plant habit, yield, and total alkaloidal content of the leaves and tops recorded. Increase in height of treated plants was due to increased internode length, especially on the treated side of the plant. Rapid growth in stem diameter caused axial splitting of the outer tissues resulting in the appearance of scar tissue. Increases in dry weights of aerial parts, observed only in plants treated over a limited period, were due to greater stem development and did not vary with the dose of gibberellic acid given. In plants treated at intervals over a long growing period, there was increased stem production but no gain in total dry weight. Yields of leaves and tops in treated plants were not significantly different from those in control groups. Significantly increased root weights were noted for a few groups of treated plants but proportion of root to total plant weight remained constant. There was a significant decrease in percentage alkaloidal content of leaves and tops in treated plants, the content being lowest in the plants which had received the highest dose of gibberellic acid

Some effects of gibberellic acid on the growth and alkaloidal content of *Datura stramonium*, *Atropa belladonna*[1,2], and *Hyoscyamus niger*[3] have been reported. In the first paper results were described for plants of *Datura stramomium* grown under greenhouse conditions and treated over a short growth period. In the present communication results are given for plants of this species grown under field conditions and treated with successive doses of gibberellic acid over intermediate and long growth periods.

EXPERIMENTAL

In 1958, plants of *D. stramonium* were raised from a singie strain of seeds obtained from Chelsea Physic Garden and in 1959 from seeds taken from a single capsule of an untreated plant grown in the previous season. Seeds were sown in March, the seedlings raised in a heated greenhouse and hardened off in a cold frame before transplanting into well worked soil in mid-June. In 1958, 100 strong plants were arranged in rows of 10, allowing 18 inches between each plant, in a plot of virgin soil to which a balanced fertiliser had been added at the rate of 200 g. per square yard. The plants were left for 1 week to establish before treatment began, then each plant was randomly assigned to one of ten groups which were treated as follows. Two groups (G and H) received 20μg., two groups (D and J) 100 μg., and two groups (A and C) 200 μg. gibberellic acid per dose per plant.

The gibberellic acid was dissolved in absolute ethanol sufficient to give the required dose in 0·002 ml. and this was injected by means of a micrometer syringe into the base of a leaf situated near the growing point on one

side of the plant. Five such treatments were given at weekly intervals. Four groups of plants (F, I, B, and E) were left as untreated controls. In each of the groups C, J, and I, one plant became stunted in growth after transplanting: these were not included in the experiment. Flowers were removed from all plants at intervals, so that no fruits were allowed to develop, since it has been shown in species of *Datura* that removal of the

FIG. 1. *Datura stramonium* L., untreated plant, x 1/8. Scale = 10 cm.

flowers leads to increased alkaloidal content in the leaves[4,5], whereas at the time of fruit and seed formation there is a gradual decrease in the amount of leaf alkaloid[6].

The first collection, of entire plants in groups G, D, A, and F, was made 1 week after the final treatment; the second collection, plants of groups H. J. C, and I, 2 weeks after the final treatment; and the third collection of untreated plants in groups B and E, was made after a further 5 weeks. Collection was made early in the day during dry weather. Each plant was handled separately, the roots carefully removed, washed, dried, and weighed. The leaves and tops were separated from the stouter main stems and branches, and the materials dried in an air oven at 55°, then weighed. In Stramonium B.P. there is a limit for the amount of stems exceeding 5 mm. width, therefore the leaves and tops only were prepared for assay by powdering, and sifting through a No. 60 sieve. The samples

were stored in well filled, screw-capped jars containing silica gel as a desiccant.

Having observed that any increased weight due to gibberellic acid treatment was negligible compared with increases resulting from extended periods of growth, it was decided to treat plants with gibberellic acid at intervals throughout the growing season to see if the stimulant effect could be prolonged. In 1959, 42 young plants were set out in a different plot from that used in 1958 and 50 g. of balanced fertiliser was worked into the soil around each plant. The plants were randomly allocated to three groups. After 1 week the following treatments were started: plants in group Y received 20 μg. doses and plants in group Z, 200 μg. doses of gibberellic acid at weekly intervals for 4 weeks, followed by three similar doses injected at fortnightly intervals. Ten days after the final injection all plants, including the untreated controls (group X), were collected at the same time, measured for height and width of aerial parts (Table I), then divided, dried, and weighed as before. The leaves and tops were powdered and assayed.

FIG. 2. *Datura stramonium* L., plant treated with gibberellic acid showing reduction in the angle of branching, x 1/8.

Assay

Samples were assayed for total alkaloidal content using the Vitali colour reaction and following the method of Jackson[7], with slight modifications.

One g. of powder was weighed into a 30 ml. beaker and stirred with 0·9 ml. of ethanol (95 per cent) and 0·1 ml. of dilute solution of ammonia. Five ml. of chloroform was added and the mixture heated just to boiling; it was then transferred to a miniature percolator, washing out the beaker with further quantities of boiling chloroform. Percolation was continued with cold chloroform until exactly 31 ml. percolate had collected. Sufficient 6 per cent solution of acetic acid (made with 5 per cent ethanol) was

added to the percolate to bring the volume to exactly 80 ml. The mixture was shaken gently for 30 seconds, allowed to separate, and 5 to 10 ml. of the acid layer filtered through dry filter paper. 0·5 ml. of the filtrate was evaporated just to dryness in a small dish over a boiling water bath, the residue thoroughly moistened with 0·2 ml. of fuming nitric acid (Analar S.G. 1·5) and immediately heated to dryness over a water bath. The nitrated residue was dissolved in about 6 ml. acetone (Analar) and transferred to a dry 10 ml. volumetric flask; 2 ml. of isopropylamine[8], then

TABLE I

EFFECT OF GIBBERELLIC ACID ON PLANT HEIGHT AND WIDTH

Group	No. of plants	Treatment	Height (cm.)	Max. width (cm.)
X	14	Controls	43 — 52·3 — 70	51 — 63·5 — 79
Y	14	7 × 20 µg. acid per plant	69 — 78·2 — 104	36 — 59·9 — 91
Z	14	7 × 200 µg. acid per plant	66 — 87·1 — 99	33 — 52·8 — 81

0·1 ml. of a freshly prepared 2 per cent solution of potassium hydroxide (Analar) in absolute methanol were added and the volume made up to exactly 10 ml. with acetone. The colour density of the solution was measured at a wavelength of 555 mµ in a 1 cm. cell of a Unicam SP. 600 spectrophotometer, using acetone as a blank, exactly 2 minutes after the addition of the potassium hydroxide since the colour is not stable. The

TABLE II

EFFECT OF GIBBERELLIC ACID ON PLANT DRY WEIGHT YIELD

	Group	No. of plants	Treatment per plant	Aerial parts		Leaves and tops		Stems		Roots	
				Mean wt. (g.)	*t*	Mean wt. (g.)	*t*	Mean wt. (g.)	*t*	Mean wt. (g.)	*t*
1st Crop 1958	F	10	Controls	10·18		8·40		1·78		1·70	
	G	10	5 × 20 µg. acid	10·51	0·21	7·96	0·34	2·55	1·90	1·63	0·27
	D	10	5 × 100 µg. ,,	12·66	1·58	9·53	0·88	3·13	3·32	2·34	2·46
	A	10	5 × 200 µg. ,,	10·38	0·13	8·02	0·30	2·36	1·43	2·02	1·23
2nd Crop 1958	I	9	Controls	13·94		10·99		2·95		2·52	
	H	10	5 × 20 µg. acid	18·66	2·08	11·71	0·57	6·95	3·86	3·16	3·11
	J	9	5 × 100 µg. ,,	18·10	1·78	11·02	0·32	7·08	3·89	3·48	4·55
	C	9	5 × 200 µg. ,,	18·86	2·11	11·61	0·50	7·25	4·05	3·30	3·70
3rd Crop 1958	B	10	Controls	70·19		47·27		22·92		11·15	
	E	10	,,	68·86		46·30		22·56		11·30	
1959 Crop	X	14	Controls	28·71		24·01		4·70		5·03	
	Y	14	7 × 20 µg. acid	28·57	0·02	20·63	1·31	7·94	2·44	6·84	2·21
	Z	14	7 × 200 µg. ,,	27·63	0·29	18·20	2·26	9·43	3·57	6·31	1·56

For P = 0·95, *t* = 2·04.

alkaloidal content of each sample was calculated from the means of duplicate or triplicate readings, using a conversion factor (K) determined from colour density figures obtained using 1 to 5 ml. quantities of 0·1 per cent chloroformic solution of atropine in place of the 1 g. samples of powder in the previously described method. $K = \frac{\text{colour density}}{\text{mg. atropine}} = 8{\cdot}2.$

The content of alkaloid per cent was calculated with reference to each sample dried to constant weight at 105°.

RESULTS

Effects of Gibberellic Acid on Morphology of Plants

All treated plants were taller than untreated controls (Fig. 1) due to greater stem development, but they were usually less spreading due to a reduction in the angle of branching (Fig. 2). Measurements of heights

FIG. 3. *Datura stramonium* L., plant treated with gibberellic acid on one side only, x 1/8.

and maximum widths for the 1959 crop, at the time of collection, are given in Table I. It was noticeable that plants injected on the same side, relative to the branching at the first node, exhibited the characters of a treated plant only on the treated side, the appearance on the non-treated

side being normal (Fig. 3). The stems on the treated side were thicker, and on the internode beyond the first fork numerous closely-arranged, axially elongated, pale brown areas of scar tissue were always present (Fig. 4). Frequently, similar but fewer, scars appeared on the higher internodes of this side; sometimes scars appeared on the main stem below

FIG. 4. *Datura stramonium* L. Scar tissue on stem of plant treated with gibberellic acid, x 1/2.

the first node, but only rarely were a few small scars observed on the non-treated side and, if present, they were very close to the first node. The scarring was due to vertical splitting of the outer tissues, often all tissues external to the xylem, as a result of rapid increase in stem width, the wounds being subsequently closed by scar tissue.

Leaves on the treated side were paler in colour, many were narrower, though not longer, than normal leaves and the margin often showed a much reduced indentation. Plants receiving the largest dose of gibberellic acid were usually the least healthy in appearance.

Effect of Gibberellic Acid on Yield of Plant Material

Table II shows the mean values for weights of total aerial parts, leaves and tops, stems, and roots. The values for t were calculated using figures obtained in analyses of variance on the weights for individual plants in each treated group compared with the weights of plants in control groups collected at the same time.

TABLE III

EFFECT OF GIBBERELLIC ACID ON ALKALOIDAL CONTENT OF LEAVES AND TOPS IN INDIVIDUAL PLANTS

Plant No.	Total alkaloid per cent				
	Group I controls	Group H 5 × 20 μg. acid per plant	Group J 5 × 100 μg. acid per plant	Group C 5 × 200 μg. acid per plant	Group B controls (late collection)
1	0·559	0·476	0·414	—	0·510
2	0·452	0·488	0·362	0·422	0·519
3	0·524	0·412	0·435	0·351	0·506
4	—	0·427	—	0·302	0·633
5	0·539	0·385	0·398	0·418	0·464
6	0·485	0·483	0·503	0·388	0·567
7	0·569	0·464	0·385	0·388	0·709
8	0·555	0·401	0·481	0·396	0·656
9	0·639	0·448	0·407	0·415	0·715
10	0·532	0·491	0·495	0·372	0·627
Mean	0·539	0·447	0·431	0·384	0·591
t		3·78	4·33	6·21	0·53

For P = 0·95, t = 2·04 except in comparison of Groups I and B where for P = 0·95, t = 2·31.

TABLE IV

EFFECT OF GIBBERELLIC ACID ON TOTAL ALKALOIDAL CONTENT OF LEAVES AND TOPS, DETERMINED ON POOLED SAMPLES

	Group	No. of plants	Treatment per plant	Alkaloid per cent
1st Crop 1958	F	10	controls	0·403
	G	10	5 × 20 μg. acid	0·330
	D	10	5 × 100 μg ,,	0·272
	A	10	5 × 200 μg. ,,	0·298
2nd Crop 1958	I	9	controls	0·537
	H	10	5 × 20 μg. acid	0·492
	J	9	5 × 100 μg. ,,	0·449
	C	9	5 × 200 μg. ,,	0·385
3rd Crop 1958	B	10	controls	0·597
	E	10	,,	0·631
1959 Crop	X	14	controls	0·809
	Y	14	7 × 20 μg. acid	0·685
	Z	14	7 × 200 μg. ,,	0·577

It can be seen that only plants of the second collection in 1958 showed significant increases in total weight of aerial parts as compared with controls, but most groups of treated plants showed significant increases in stem weight. The groups with increased total weight yielded approximately the same weight of leaves and tops as controls. Other groups, including those treated over a prolonged period in 1959, showed decrease in weight of leaves and tops but the only statistically significant reduction was found in plants which received the high dose of gibberellic acid in

1959 (Group Z). The late collected controls of the 1958 crop showed great weight increases over all groups collected earlier, but it is interesting to note that the proportion of stem to total weight in these older plants, while higher than in the early collected controls, was lower than in any group of treated plants.

The effect on root weight was variable, there being significant increases in some, but not all, treated groups in the three crops examined. However the ratio of root weight to total plant weight remained fairly constant.

Effect of Gibberellic Acid on Total Alkaloidal Content of Leaves and Tops

For groups C, J, H, I, and B the leaves and tops from each plant were assayed separately and the results (Table III) treated statistically. The results for the first four groups were analysed for variance and the value of t calculated. A significant reduction in alkaloidal content was observed in each group of treated plants, as compared with the control group collected at the same time, the content falling with increase in dose of gibberellic acid given. The percentage of alkaloid was not significantly different in the two groups of untreated plants although the total production per plant was much greater in the late collected controls (Group B) since these showed a considerable increase in leaf yield.

For these and the remaining groups pooled samples of leaves and tops were assayed and the results are given in Table IV. It was again evident that gibberellic acid treatment had caused a loss in alkaloid yield.

Discussion

Increases in plant height, due to increased internode length, and chlorosis of leaves resulting from gibberellic acid treatment are well known for many plants, including *D. stramonium*[1], and these effects have been confirmed for this species.

By injecting this growth-promoting substance the dosage could be carefully controlled and the site of treatment selected, two advantages as compared with the application of an aqueous spray. It is known that gibberellic acid is quickly translocated from the point of application to other parts of the plant, but it is of interest to note that in this dichasially branched species only the treated side of the plant appeared to be affected. Splitting of the outer stem tissues was an effect of treatment and from these observations it appears that gibberellic acid passes from the apex of the treated stems down to the main stem, but not across to the untreated side of the plant.

Dry weight figures confirm that gibberellic acid treatment causes increased stem production but the present results conflict with an earlier report[1] that treatment gives increases in yield of leaves and tops and a decrease in root weight for stramonium. It appears that total weights of aerial parts can be increased by a limited number of treatments with gibberellic acid but further doses do not continue the stimulus to increased growth throughout the growing season.

Confirmation is given that treatment causes significant reduction in total alkaloidal content of leaves and tops[1]. The higher the dose of

gibberellic acid given the greater the effect; the lower doses caused about 20 per cent reduction and the higher doses about 30 per cent reduction in the proportion of alkaloid present, as compared with untreated controls.

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