Effect of Various Concentrations and Frequency of Application of Gibberellic Acid on Growth and Formation of Metabolic Products of *Datura stramonium*

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In a 2 × 4 × 5 factorial experiment employing 320 plants in randomized blocks with eight replications the following salient points were noted from a statistical analysis of the data: the greatest increase in height was induced with the 192-mcg. dosage of giberellic acid (GA). All treated plants at the 12, 48, and 192-mcg. dosages of GA grew more rapidly than controls. Height increases were significantly different at each dosage level. The most favorable response on height was between the 12-mcg. and 48-mcg. levels. The total dry weights of treated plants were significantly affected by dosage and age. The growth rate varied with the dose of GA used. The frequency of application (whether one or two installments were given weekly) did not significantly affect the aforementioned variables. Analyses of pooled samples of treated plants indicated the following general trends: reduced concentration of alkaloids in the plant organs, greatest reductions usually noted at the higher dosage levels, decreased per cent of chlorophyll, marked increases in the total sugars and starch content of the leaf-tops, considerable decreases in the petroleum ether extract of the leaf-tops, and qualitative differences in the ether extract of the leaf-tops.

THE METABOLIC EFFECTS of gibberellic acid (GA) on many plants (1–4) and with some medicinal plants (5) have been described. Among the members of the Solanaceae which have been investigated are *Datura stramonium* (6–8), *D. innoxia* (9), *D. meteloides* (10), *Atropa belladonna* (6, 7, 11, 12), *Hyoscyamus niger* (13), and tobacco (14–16). Gibberellin treatment generally induced a reduction in the concentration of alkaloids in the aerial parts of the plants and increases in plant height and stem weight. Variable results were noted on root growth and root alkaloid production (5–16)

The objectives of this research were: (*a*) to determine the concentration of GA which would produce the most favorable response on growth; (*b*) to determine whether frequency of application of the chemical, *i.e.*, whether a given dosage given once weekly *versus* one-half dose given twice weekly, would elicit different responses on growth; (*c*) to submit all of the data obtained during the growth phase to statistical analysis in order to determine which variables were statistically significant; (*d*) to ascertain whether GA treatment would alter the quantity of various metabolic products produced by the plant

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EXPERIMENTAL

Procedure.—*Datura stramonium* plants employed in this study were grown under greenhouse conditions. Seeds obtained from a single plant grown in the Oregon State University drug garden in 1958 were germinated in flats containing a soil mixture of two parts of loam and one part of sand. About 100 Gm. of complete organic fertilizer[1] was incorporated into the soil of each flat. On June 15, 1959, 350 thirty-five-day-old seedlings were transplanted into individual 1-gallon metal containers that had been filled with the sandy loam mixture. Approximately 4 Gm. of complete fertilizer had been thoroughly mixed into the soil of each container prior to transplantation. At zero time on June 23, 320 of the most uniform plants were selected for this study.

Explained statistically this was a 2 × 4 × 5 factorial experiment in randomized blocks with eight replications. The entire experiment employed 320 plants. The first factor was the number of installments used in applying the GA. The same dosage may be applied once weekly or in two installments twice weekly, half a dose each time. Therefore, this factor had two levels. The second factor had four levels, namely, 0, 12, 48, and 192-mcg. dosages of GA. The third factor was the age of the plants at the time of harvesting. This factor had five levels, namely, 0, 1, 2, 3, and 4 weeks. Each replication included plants which were about the same height.

At zero time (June 23) height and leaf measurements were made, treatment with GA was given to the designated randomized plants, and the eight replications from each of the eight series were harvested. The harvested plants were divided into three portions—leaf-tops, stems, and roots. The plant parts were cut into small segments; fresh weights were taken immediately. The plants were

[1] Organic Morcrop, Chas. Lilly Co., Seattle, Wash. (Analysis—5% total nitrogen, 3% available phosphate, 2% available potash.)

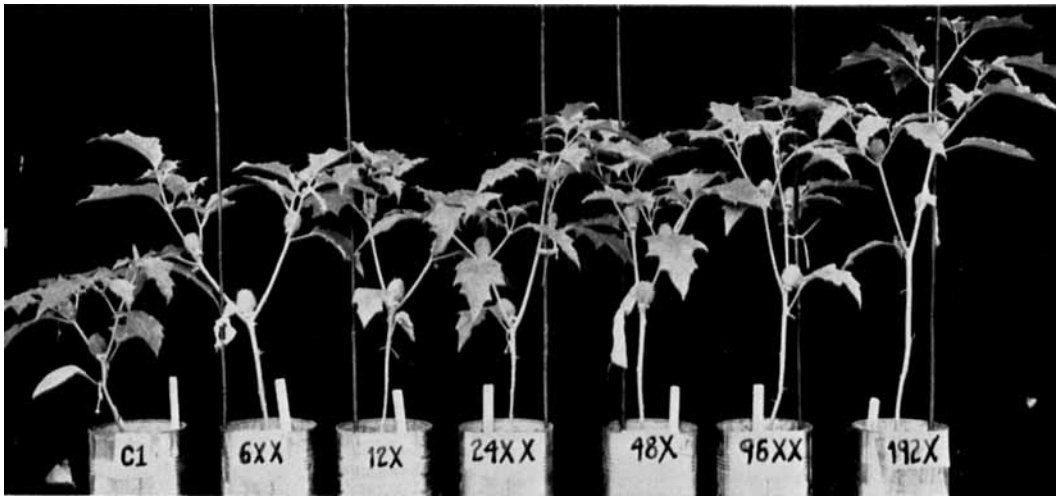

Fig. 1.—Effect of GA-treatments on the growth of *Datura stramonium*. Key = C_1, control; 6XX, 6 mcg. twice weekly; 12X, 12 mcg. weekly; 24XX, 24 mcg. twice weekly; 48X, 48 mcg. weekly; 96XX, 96 mcg. twice weekly; 192X, 192 mcg. weekly. Plants at the final harvest were about 10 weeks old.

then dried for 48 hours at 48.5° in a circulating hot-air drier and were stored in a desiccator until dry weight was determined. At this time the parts were pooled according to treatment and pulverized to a No. 40 powder in the Wiley mill. However, an exception to this procedure was made at the first harvest (zero time) when all samples were pooled and designated as controls, since treatment had not begun with the zero harvest series. This was done to assure a sufficient quantity of plant material for subsequent analytical work. The powder was stored in colored glass containers and a desiccator for subsequent analyses. This same procedure was followed at each week thereafter until all plants were harvested by the end of the 4-week treatment period. The day temperature in the greenhouse during the 4-week observation period was often high, frequently ranging from 100–110° F. for several hours in the afternoon.

Height and leaf measurements were taken three times weekly during the treatment period. All plants were dusted lightly thrice weekly with a 4% malathion dust. Aqueous solutions of GA[2] were prepared fresh each treatment day from a stock alcoholic solution of the chemical. The calculated dosage, in 0.02-ml. portions, was delivered from a micropipet onto the surface of the youngest unfounding leaf. The groups of plants which were designated to be treated twice weekly received the second weekly treatment 3 days after the first dosage was applied.

Growth Effects.—Characteristic gibberellin effects were noted in the treated plants (Fig. 1). There was marked elongation at the internodes which was more evident with plants receiving the higher doses of GA. The leaves of treated plants appeared chlorotic, narrower, and thinner. Abscission of the lower leaves of treated plants was more rapid than in the controls. The stems of the controls at the final harvest appeared more succulent and less woody than those of treated plants. Furthermore, a higher per cent of fruit-set was noted in the control group. All treated plants were taller than controls (Fig. 2). The greatest increase in height was noted in the plants receiving the largest dose (192 mcg.) of GA. This group was 73% taller than controls at the final harvest.

Fresh and Dry Weights.—Mean dry weight data indicated that stem growth of treated plants was generally significantly increased throughout the 4-week observation period (Table I). About a two-fold increase or more was noted in the stems of all treated plants at the end of the second week. At the final harvest the 192-mcg. series demonstrated the highest stem weight, 217% that of controls. It appeared that maximal stimulation of stem growth was attained at the end of 2 weeks. Variable results were noted in leaf-tops and root weights during

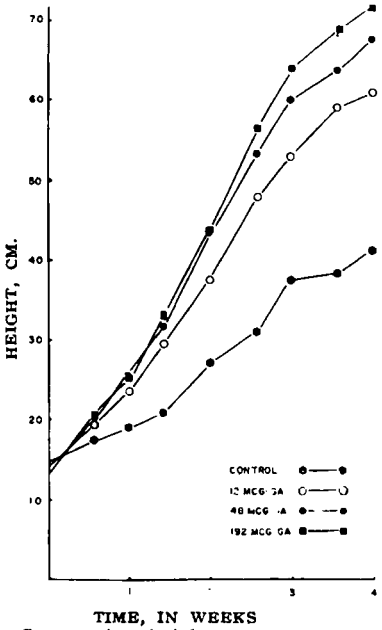

Fig. 2.—Stramonium height measurements. Plants were about 6 weeks old at zero time.

[2] The gibberellic acid employed in this study was furnished through the courtesy of Dr. Edwin F. Alder, Head of Plant Science Research, Eli Lilly and Co., Greenfield, Ind.

TABLE I.—MEAN WEIGHTS OF STRAMONIUM PLANT PARTS

Treatment mcg.	Harvest Time, wk.	Total Weight			Leaf-Tops			Stems			Roots		
		Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %	Fresh, Gm.	Dry, Gm.	Control Dry Wt., %
Control[a]	0	5.29	0.539	...	2.18	0.306	...	1.27	0.123	...	1.84	0.134	...
12	0	5.00	0.498	92.3	2.15	0.280	91.5	1.06	0.100	81.3	1.80	0.117	87.3
6 (2×)	0	6.04	0.577	107.0	2.67	0.317	103.5	1.43	0.131	106.5	1.94	0.130	97.0
48	0	4.44	0.451	83.6	1.88	0.248	81.0	1.09	0.095	77.2	1.48	0.108	80.5
24 (2×)	0	5.09	0.555	102.9	2.05	0.282	92.1	1.17	0.116	94.3	1.87	0.157	117.1
192	0	4.76	0.491	91.0	2.21	0.286	93.4	1.32	0.125	101.6	1.23	0.080	59.7
96 (2×)	0	5.00	0.529	98.1	2.28	0.297	97.0	1.36	0.132	107.3	1.36	0.100	74.6
Control[a]	1	14.02	1.133	...	6.92	0.680	...	3.25	0.242	...	3.86	0.211	...
12	1	15.15	1.235	109.0	6.75	0.713	104.8	3.95	0.260	107.4	4.46	0.262	124.1
6 (2×)	1	12.33	0.999	88.1	5.04	0.584	85.8	3.33	0.220	90.9	3.96	0.195	92.4
48	1	14.11	1.269	112.0	5.95	0.699	102.7	4.55	0.301	124.3	3.61	0.269	127.4
24 (2×)	1	12.14	1.029	90.8	5.21	0.554	81.4	3.74	0.264	109.0	3.19	0.211	100.0
192	1	15.43	1.260	111.2	6.95	0.721	106.0	4.88	0.312	128.9	3.58	0.227	107.5
96 (2×)	1	14.74	1.158	102.2	6.93	0.710	104.4	4.66	0.301	124.3	3.15	0.192	90.9
Control[a]	2	30.97	2.644	...	17.92	1.708	...	6.77	0.480	...	6.28	0.445	...
12	2	38.55	3.139	118.7	18.09	1.739	101.8	12.07	0.868	180.8	8.40	0.532	119.5
6 (2×)	2	32.33	2.720	102.8	15.62	1.600	93.6	10.41	0.770	160.4	6.19	0.350	78.6
48	2	34.30	3.540	133.8	18.50	1.970	115.3	14.10	1.180	245.8	6.71	0.390	87.6
24 (2×)	2	36.96	3.131	118.4	15.74	1.656	96.9	12.66	0.927	193.1	8.58	0.548	123.1
192	2	41.29	3.601	136.1	18.73	1.992	116.6	14.69	1.058	220.4	7.87	0.551	123.8
96 (2×)	2	37.57	3.644	137.8	15.77	1.799	105.3	14.10	1.348	280.8	7.70	0.497	111.6
Control[a]	3	56.11	7.345	...	30.74	4.338	...	14.15	2.008	...	11.22	0.996	...
12	3	62.67	8.294	112.9	30.78	4.433	102.1	21.48	2.845	141.6	10.41	1.015	101.9
6 (2×)	3	63.44	7.526	102.4	30.88	4.029	92.8	20.21	2.524	125.6	12.36	0.973	97.6
48	3	64.54	8.175	111.3	29.36	4.071	93.8	24.27	3.246	161.6	10.91	0.870	87.3
24 (2×)	3	55.18	7.358	100.1	23.77	3.363	77.5	22.32	3.167	157.7	9.09	0.828	83.1
192	3	60.37	8.172	111.2	28.32	4.017	92.6	23.55	3.430	170.8	8.50	0.725	72.7
96 (2×)	3	59.84	7.946	108.1	25.46	3.573	82.3	25.92	3.668	182.6	8.28	0.705	70.7
Control[a]	4	70.84	11.24	...	40.56	6.82	...	16.87	3.35	...	13.41	1.05	...
12	4	71.63	12.92	114.9	35.22	6.16	90.3	24.84	5.39	160.8	11.57	1.37	130.4
6 (2×)	4	76.87	14.21	126.4	41.82	7.42	108.7	24.45	5.39	160.8	10.59	1.40	133.3
48	4	69.68	13.52	120.2	32.62	5.84	85.6	28.95	6.53	194.9	8.11	1.41	134.2
24 (2×)	4	81.48	14.21	126.4	39.95	6.92	101.4	26.45	5.80	173.1	15.07	1.49	141.9
192	4	85.27	15.38	136.8	38.81	6.53	95.7	32.69	7.28	217.3	13.77	1.57	149.5
96 (2×)	4	69.73	12.44	110.6	31.34	5.50	80.6	26.34	5.65	168.6	12.05	1.29	122.8

[a] Average of two control groups of eight plants each.

TABLE II.—TOTAL ALKALOID CONTENT[a] OF STRAMONIUM PER PLANT AND ORGAN

Treatment, mcg.	Harvest Time, wk.	Per Plant		Leaf-Tops		Stems		Roots	
		mg.	Control, %	mg.	Control, %	mg.	Control, %	mg.	Control, %
Control[b]	0	1.07	...	0.58	...	0.33	...	0.16	...
Control[b]	1	3.08	...	2.10	...	0.87	...	0.11	...
12	1	2.29	74.3	1.35	64.2	0.65	74.7	0.29	263.6
6 (2×)	1	1.79	58.1	0.99	47.1	0.57	65.5	0.23	209.0
48	1	1.82	59.1	1.05	50.0	0.61	70.1	0.16	145.4
24 (2×)	1	1.48	48.0	0.88	41.9	0.47	54.0	0.13	118.1
192	1	1.95	63.3	1.22	58.0	0.59	67.8	0.14	127.2
96 (2×)	1	1.65	53.5	0.99	47.1	0.51	58.6	0.15	136.3
Control[b]	2	5.69	...	4.28	...	1.01	...	0.40	...
12	2	5.63	98.9	3.50	81.7	1.74	172.2	0.39	97.5
6 (2×)	2	4.64	81.5	3.04	71.0	1.39	137.6	0.21	52.5
48	2	6.18	108.6	4.14	96.7	1.65	163.3	0.39	97.5
24 (2×)	2	5.08	89.2	3.15	73.5	1.49	147.5	0.44	110.0
192	2	4.45	78.2	2.99	69.8	0.85	84.1	0.61	152.5
96 (2×)	2	4.56	80.1	3.06	71.4	0.95	94.0	0.55	137.5
Control[b]	3	10.26	...	7.95	...	1.61	...	0.70	...
12	3	12.02	117.1	8.42	105.9	2.99	185.7	0.61	87.1
6 (2×)	3	9.61	93.6	6.85	86.1	2.27	140.9	0.49	70.0
48	3	9.07	88.4	5.70	71.6	2.93	181.9	0.44	62.9
24 (2×)	3	8.34	81.2	5.38	67.6	2.54	157.7	0.42	60.0
192	3	9.35	91.1	7.74	97.3	1.37	85.0	0.24	34.2
96 (2×)	3	8.22	80.1	6.07	76.3	1.84	114.2	0.31	44.2
Control[b]	4	18.93	...	14.19	...	3.69	...	1.05	...
12	4	24.69	130.4	17.25	121.5	5.93	160.7	1.51	143.8
6 (2×)	4	26.35	139.1	20.03	141.1	4.85	131.4	1.47	140.0
48	4	20.38	107.6	12.85	90.5	6.40	173.4	1.13	107.6
24 (2×)	4	21.57	113.9	13.15	92.6	7.08	191.8	1.34	127.6
192	4	21.78	115.0	14.69	103.5	5.72	155.0	1.37	130.4
96 (2×)	4	17.88	94.4	12.10	85.2	4.52	122.4	1.26	120.0

[a] Alkaloid content for plant parts calculated from dry weight and alkaloid analyses data; total plant alkaloids = leaves and tops + stems and roots; based on av./plant/groups. [b] Average of two control groups of eight plants each.

the first 3 weeks. However, at the fourth week considerable increases in the weights of the roots of the treated plants were noted (Table I). Variable results were noted in the total dry weight of treated plants the first week, but increased growth (dry wt.) was observed thereafter.

Analysis for Alkaloids.—One-gram samples of the dried plant parts, employing pooled samples, were assayed for total alkaloids according to the Witt-Youngken method (17), substituting chloroform for benzene as the immiscible solvent. A minimum of three duplicate determinations was carried out on each sample. All of the treatments induced significant reduction in the concentration of alkaloids in the leaf-tops during the first 2 weeks. Generally, the leaf-tops of the treated plants had decreased concentrations at the third week, whereas increased concentrations were noted at the fourth week. The stems of the treated plants generally indicated markedly decreased concentration of alkaloids. The stronger treatment (192-mcg. dose) generally induced the greatest reduction. Variable results were noted in the roots of the treated plants. The general trend was a higher concentration than controls during the first 2 weeks and a lower concentration the last 2 weeks.

Total Plant Alkaloids.—The total alkaloids per plant and per plant organ were obtained by multi-

TABLE III.—CHLOROPHYLL *a* AND *b* CONTENT[a] OF STRAMONIUM AERIAL PARTS

Treatment, mcg.	Harvest Time, wk.	Leaf-Tops			Stems		
		Chl. *a*	Control, %	Chl. *b*	Chl. *a*	Control, %	Chl. *b*
Control	0	0.167	...	0.087	0.0075	...	0.0055
Control	1	0.274	...	0.193	[b]	[b]	[b]
12	1	0.268	98.0	0.165	[b]	[b]	[b]
48	1	0.228	83.2	0.169	[b]	[b]	[b]
192	1	0.273	99.6	0.194	[b]	[b]	[b]
Control	2	0.286	...	0.191	0.021	...	0.018
12	2	0.259	90.5	0.157	0.014	67	0.011
48	2	0.225	78.6	0.198	0.010	48	0.009
192	2	0.230	80.4	0.122	0.018	86	0.016
Control	3	0.276	...	0.200	0.010	...	0.0084
12	3	0.187	67.7	0.109	0.010	100	0.0070
48	3	0.196	71.0	0.102	0.013	130	0.0065
192	3	0.251	93.5	0.153	0.014	140	0.011
Control	4	0.165	...	0.058	0.0056	...	0.0044
12	4	0.043	26.1	0.028	0.0052	93	0.0035
48	4	0.056	34.5	0.039	0.0046	82	0.0038
192	4	0.063	38.2	0.043	0.0043	77	0.0028

[a] Results expressed as per cent of dry wt.; 1-Gm. samples for leaf-top determinations; 2-Gm. samples for stems. [b] Insufficient material remained for chlorophyll determinations.

TABLE IV.—SELECTIVE SOLVENT EXTRACTIONS[a] OF STRAMONIUM LEAF-TOPS

Harvest Time, wk.	Treatment: Control	48 mcg.	192 mcg.
	Petroleum Ether		
0	1.44[b]	1.44[b]	1.44[b]
1	1.13	0.58	0.98
2	2.21	1.59	1.74
3	4.81	4.10	2.95
4	5.70	3.38	2.46
	Ether		
0	0.81[b]	0.81[b]	0.81[b]
1	0.75	0.55	0.53
2	1.13	0.95	1.09
3	0.84	1.09	0.83
4	0.85	1.05	1.12

[a] Expressed as per cent of dry wt.; based on 1-Gm. samples. [b] Pooled sample of 64 plants from all eight series.

TABLE V.—TOTAL SUGAR[a] AND STARCH[b] CONTENT OF STRAMONIUM LEAF-TOPS

Treatment, mcg.	Harvest Time, wk.	Total Sugars: mg./Gm.	Control, %	Starch: mg./Gm.	Control, %
Control	0	33.7[c]	...	7.33[c]	...
Control	1	14.9	...	3.41	...
48	1	17.9	120.	3.92	115.
192	1	37.9	254.	2.51	74.
Control	2	32.5	...	4.60	...
48	2	15.0	46	6.99	141.
192	2	32.1	99.	7.11	155
Control	3	21.1	...	9.76	...
48	3	28.8	184.	10.24	105.
192	3	42.5	201.	11.37	116.
Control	4	22.8	...	8.58	...
48	4	59.0	259.	10.67	124.
192	4	56.0	246.	13.36	156.

[a] Method of McCready (20). [b] Method of Viles and Silverman (22). [c] Pooled sample of 64 plants from all eight series.

plying the dry weight of the plant part by the per cent of alkaloids obtained from the alkaloid analysis and expressing the results in milligrams (Table II).

The total alkaloid content per treated plant was generally markedly reduced during the first 3 weeks but increased at the final harvest. The favorable effect the fourth week was due primarily to increased growth in the treated plants. The leaf-tops of the treated plants indicated a similar trend, except that both decreased and increased content were noted at the terminal harvest. On the other hand, the content of alkaloids in the stems was significantly decreased the first week but usually increased the remaining 3 weeks. Variable results were noted in total root alkaloids.

Chlorophyll Content.—The chlorophyll analysis was conduted by a method described by Gjerstad (18), but modified by employing dried material instead of fresh samples and using a Soxhlet extraction apparatus. A minimum of two duplicate determinations was carried out on each sample. The chlorophyll was completely extracted from the powdered material with a 20% water-in-acetone solvent. The results of the chlorophyll analysis are shown in Table III. The chlorophyll content of the treated plants was markedly reduced in the treated plants. The decreases of chlorophyll *a* content in the leaves of the treated plants at the final harvest were considered significant. Surprisingly, at this harvest, the group treated with the lowest concentration of GA (12 mcg.) contained the least amount of chlorophyll *a*. The amount of stem chlorophyll, except at the third harvest, was considerably less in the treated plants. The ratio of chlorophyll *a* to *b* was somewhat higher in the stems than in the leaves. The decreased content of chlorophyll in the leaves confirms our observation that the leaves of treated plants appeared chlorotic.

Selective Solvent Extraction.—To determine the effect of gibberellin treatment on the formation of other types of metabolic products, 1-Gm. samples of the untreated, 48-mcg., and 192-mcg. groups were completely extracted with petroleum ether and then with anhydrous ether. The method of Dragendorff (19) was employed with use of the Soxhlet extractor. At least two duplicate determinations were carried out on each sample. The amount of petroleum ether extractive was considerably less in the treated plants at each harvest (Table IV). The decreases of 41% in the 48-mcg. group and of 57% in the 192-mcg. group at the final harvest were considered significant. The lower dosage of GA caused a greater reduction during the first 2 weeks. On the other hand, the plants treated with 192 mcg. of GA displayed greater decreases at the latter two harvests (Table V). The content of ether extractive in the treated plants was considerably less than controls at the first two harvests and somewhat greater than controls at the final harvest. Of greater importance was the apparent qualitative difference in the ether soluble extractives of the treated plants. The waxy residue from the controls was dark green in color, whereas that from the treated plants was a yellowish brown color.

Carbohydrate Content.—The procedure for the extraction of total sugars was adapted from that of McCready (20). A 250-mg. sample of leaf material

TABLE VI.—ANALYSIS OF VARIANCE FOR GROWTH DATA

Source of Variation	Degrees of Freedom	Mean Square: Height	Total Dry Wt.	Leaf-Tops Dry Wt.	Stem Dry Wt.	Root Dry Wt.
Replication	7	192.36	26.26[a]	13.90[a]	2.75[a]	0.09
Installment	1	84.46	4.69	0.63	1.37	0.07
Dosage	3	4,049.34[a]	12.23[a]	1.09	18.05[a]	0.08
Age	4	24,826.19[a]	1,796.09[a]	432.04[a]	310.89[a]	16.17[a]
Ins. × Dosage	3	0.23	1.50	0.79	0.12	0.05
Ins. × Age	4	26.25	0.81	1.32	1.37[a]	0.03
Dosage × Age	12	568.97[a]	4.62[a]	0.74	5.28[a]	0.20[a]
Ins. × Dos. × Age	12	13.57	2.55	1.02	0.58	*0.04*
Error	273	36.53	2.48	0.95	0.49	0.05
Total	319					

[a] Significant at $p = 0.05$.

was shaken with 15 ml. of hot, neutral (to litmus), 80% ethanol for 30 minutes on a wrist-action shaker. The mixture was centrifuged and the supernatant liquid decanted into a 100-ml. volumetric flask containing 25 ml. of 15% neutral lead acetate solution. The residue was washed three times with ethanol solution and centrifuged. Each time the supernatant liquid was decanted into the flask containing the lead acetate solution. Powdered sodium oxalate was added to remove the lead ions and the mixture was brought to the 100-ml. mark with distilled water. The mixture was centrifuged and aliquots taken from the supernatant liquid. Total sugar was determined by the anthrone–sulfuric acid color reaction (21) employing 0.5-ml. aliquots of the clarified extract. The residue from the sugar extraction procedure was extracted and analyzed for starch (amylose) according to the method of Viles and Silverman (22). The results of these analyses are presented in Table VI.

The gibberellin treatments induced considerable increases in the total sugar content of the plants, except at the second week (Table VI). The increased content in the treated groups at the third and fourth week was considered significant. Marked increases in the starch content of treated plants were noted in the treated plants from the second through the fourth week.

STATISTICAL ANALYSIS OF GROWTH DATA[3]

Analysis for Height.—The analysis of variance for the height data is given in Table VI which shows that only dosage, age, and dosage times age interaction are significant. This indicates that both dosage and age affect the heights of plants. The significant interaction indicates that the rate of growth varies with dosage.

The number of installments does not affect the height of plants. Whether a certain dose is applied all at once or in two installments with half a dose each time will not make a difference on the average heights of plants.

Since dosage times age interaction was significant, a more detailed analysis of the growth rates of plants at various doses was made. The mean heights are shown in Fig. 2. The analysis of variance is shown in Table VII.

The Table shows that for control and 12-mcg. groups the deviation from linearity is not significant, while that for 48 and 192-mcg. groups is significant. This implies that the growth curves for control and 12 mcg. are approximately linear, while those for 48 and 192 are not. The average growth rates, b_1 and b_2, for control and 12 mcg. are 7.16 and 12.27 cm. per week, respectively. This difference is significant (Table VII). In other words, the plants receiving 12 mcg. of GA grow faster than control plants. For the two higher doses, the average rates of growth cannot be compared, because the growth curves cannot be approximated by straight lines. To determine whether these two curves are identical, the dosage times age interaction for 48 and 192 mcg. was tested. This interaction is not significant. The implication is that the two growth curves are parallel.

[3] The author acknowledges the assistance of Dr. Jerome C. R. Li, Chairman of Department of Statistics, Oregon State University, for assisting in the experimental design and performing the statistical analysis of the growth data.

TABLE VII.—ANALYSIS OF VARIANCE FOR GROWTH RATE FOR HEIGHT

Source of Variation	Degrees of Freedom	Mean Square	F
Control			
Linear regression	1	8,203.93	
Deviation from linearity	3	65.87	1.80
12 mcg.			
Linear regression	1	24,090.92	
Deviation from linearity	3	79.99	2.19
24 mcg.			
Linear regression	1	33,857.85	
Deviation from linearity	3	239.77	6.56[a]
192 mcg.			
Linear regression	1	38,146.06	
Deviation from linearity	3	225.57	6.17[a]
Control and 12 mcg.			
b_1 *vs.* b_2	1	2,088.99	57.19[a]
48 and 192 mcg.			
Dosage × age	4	21.01	0.58
Error	273	36.53	

[a] Significant at $p = 0.05$.

From the analyses of variance (Tables VI and VII), one can draw the following conclusions. (*a*) For the same amount of GA applied, whether one or two installments is used, the height of the plant is not affected. (*b*) A larger dose of GA will make the plants grow faster in height, but the effect of dosage is limited. The growth curves for 48 mcg. and 192 mcg. are not significantly different.

Analysis of Dry Weights of Leaf-Tops.—Only the age of the plants affect the dry weight of leaves and tops. Dosage and the number of installments are not significant (Table VI).

Analysis of Stem Dry Weights.—Table VI shows that both dosage and age effect the dry weight of stems. Significant dosage times age interaction indicates that the growth rate varies with the dosage of GA. The growth rate also varies slightly with the number of installments used in applying the acid. The conclusions can also be observed from Table I. The analysis of growth rate given in Table VIII indicates that the weight of stems grows faster with a higher dosage of GA, but the two higher doses, 48 and 192 mcg., have the same growth rate. This conclusion is also true for the height of plants.

Analysis of Root Dry Weights.—The dry weight of roots is not significantly affected by the dosage of GA or the number of installments. The roots do develop with age. The rate of growth of the roots, however, varies slightly with the dosage as indicated by the significant dosage times age interaction shown in Table VI. The analysis of the growth rate is

TABLE VIII.—ANALYSIS OF GROWTH RATE, DRY WEIGHT OF STEMS

Source of Variation	Degrees of Freedom	Mean Square	F
Dosage × Age	12	5.28	10.87[a]
0 and 12 mcg.	4	5.74	11.81[a]
48 and 192 mcg.	4	0.27	0.56
Low *vs.* High	4	9.84	20.24[a]
Error	273	0.48	

[a] Significant at $p = 0.05$.

TABLE IX.—ANALYSIS OF VARIANCE FOR GROWTH RATE, DRY WEIGHT OF ROOTS

Source of Variation	Degrees of Freedom	Mean Square	F
Dosage × Age	12	0.20	4.46[a]
Among Treated	8	0.08	1.89
Treated *vs.* Untreated	4	0.43	9.61[a]
Error	273	0.04	

[a] Significant at $p = 0.05$.

shown in Table IX. The growth rates of the roots are not significantly different among the treated plants, 12, 48, and 192 mcg. The control plants have a slightly different growth rate.

Analysis of Total Dry Weight.—Table VI indicates that the total dry weight is not affected by the number of installments, but is significantly affected by dosage and age. Significant interaction between dosage and age indicates that the growth rate varies with dosage. These conclusions can also be observed from Table I. The analysis for growth rate is given in Table X which shows that the treated plants (*i.e.*, those of the 12, 48, and 192-mcg. groups) have the same growth rate in total dry weight, but the control plants grow at a slower rate.

Statistical analysis of leaf growth data (such as leaf-blade width, leaf-blade length, and petiole length) did not indicate significant results from the dosage applied. It was noted, however, that the leaves of treated plants grew at a different rate than controls.

DISCUSSION AND CONCLUSIONS

The treated plants displayed characteristic gibberellin effects such as taller and spindlier plants, greater internodal elongation, the appearance of chlorotic leaves, and significantly increased stem growth. These effects have been reported in other solanaceous alkaloid-producing plants (5–16). Other effects generally noted in the treated plants included less succulent shoots, woodier stems, less fruit-set than controls, and a more rapid abscission of the lower leaves. There is a correlation between the statistical analysis of height data and stem dry weight data, in that the significant factors were dosage, age, and dosage times age interactions. All treated plants grew more rapidly than controls; the height and stem weights were significantly different at each dosage level; and the rate of growth varied with the dosage. The stem dry weight was the only variable significantly affected by the number of installments. The statistical analyses of both variables indicated that the most favorable response on growth was induced by the GA between the 12 and 48-mcg. dosage levels. Previously unpublished work performed in our laboratory on stramonium which was treated with weekly doses of 10, 25, 50, and 100-mcg. doses of GA indicated that the most favorable response on height was induced with the 50-mcg. dose. Thus, it was concluded that the most beneficial response on growth was near the 48-mcg. weekly dosage level.

TABLE X.—ANALYSIS OF VARIANCE FOR GROWTH RATE, TOTAL DRY WEIGHT

Source of Variation	Degrees of Freedom	Mean Square	F
Dosage × Age	12	4.62	1.86[a]
Among Treated	8	0.39	0.16
Treated *vs.* Untreated	4	13.08	5.27[a]
Error	273	2.48	

[a] Significant at $p = 0.05$.

A study of dry weight data (Table I) indicates that treatments at all dosage and frequency levels resulted in increased total plant weight at the terminal harvest. At this time reduced leaf-tops weight was generally noted; significantly increased stem weight (with over a twofold increase in the 192-mcg. group) and significantly increased root weight were found. The largest increase in stem weights were induced with the higher concentrations of GA and correlates with the height increases. A similar trend was found in the root weights. The reduced leaf-tops weight was most likely due to two factors: more rapid abscission of the lower leaves than in the controls, and less fruit-set which resulted in fewer capsules being formed in the treated plants. Generally, stem growth was significantly increased in the treated groups throughout the 4-week observation period. Variable results were noted in the weights of the other organs during the first 3 weeks. Conclusions regarding plant growth were that generally a favorable response on growth was induced by the treatments, the increased stem growth was considered significant, treated plants grew at a more rapid rate than controls, and the growth rate generally varied with the dose of GA applied.

The general trend was a reduced concentration of alkaloids in the organs of the treated plants. The largest decreases were usually noted with the higher dosage levels of GA. However, this was not true in all cases. When using the single weekly doses of 12, 48, and 192-mcg. as a basis, it was found that reduced concentrations of alkaloids were induced in the leaf-tops of these groups at the first two harvests, while increased concentrations were noted at the final harvest. A similar trend was observed in the concentration of stem alkaloids, except that significant reductions were noted at all four harvests in the 192-mcg. series. The reductions in the stems were attributed to dilution of the alkaloids which resulted from markedly increased stem growth. The roots generally demonstrated increased concentrations of alkaloids at the latter two harvests. The general trend of reduced concentration of alkaloids in the aerial parts of gibberellin-treated plants is in agreement with other reports in the literature (5–16). However, increased concentration of alkaloids such as that found in the aerial parts at the terminal harvest has not been previously reported for GA-treated plants. Brummett (23) found that, when stramonium was administered a single 100-mcg. dose of GA, significantly reduced concentrations of alkaloids occurred in all the plant organs at the first and second-week harvests. On the other hand, slight increases were found in the leaf-tops and roots at the fourth-week harvest. Brummett's plants were approximately the same age during the treatment and harvest periods as those employed in this experiment. This suggests that mature plants do not respond to

GA in the same manner as do younger plants. Further, the dosage per unit of plant weight was about twentyfold greater at the time of initial treatment than at the terminal harvest. This dilution of about $^1/_{20}$ in dosage, which was due to the increased weight of the mature plants, could also account for the increased concentration of alkaloids in the aerial parts of the treated plants at the terminal harvest.

The total alkaloid content per plant was generally considerably less in the treated groups at the first three harvests. This was due mainly to lower concentrations in most of the plant organs and a general reduction in leaf-tops and root growth. This trend was reversed at the final harvest when marked increases were usually found in the treated groups. The increases were due to a favorable effect on growth by the GA treatments and a higher concentration of alkaloids in the leaf-tops of the treated plants.

The per cent of chlorophyll in the leaf-tops of the 12, 48, and 192-mcg. groups was considerably less than controls throughout. The decreases (compared with controls) at the final harvest of 74, 65, and 62%, in the corresponding groups, were considered significant. The reduced per cent of chlorophyll in the aerial parts of treated stramonium confirms our observations in this experiment and our previous publications (5–7) that the leaves of treated plants appeared chlorotic. This is also in agreement with that reported for *Datura meteloides* (10) and spearmint (24), but does not agree with the results reported for foxglove (25) and peppermint (26).

Calculations were made from the results of the chlorophyll analysis and from the dry weight data to indicate the milligrams of chlorophyll *a* present in the leaf-tops of the plants. The average per control plant was 1.84, 4.96, 12.15, and 11.59 mg. at the first, second, third, and fourth harvests, respectively. This indicates that the maximal chlorophyll content occurred at the third harvest. Only slight differences compared with controls were found in the treated groups at the first and second harvests. However, the chlorophyll *a* content was markedly reduced by the GA-treatment at the third and fourth harvests. The figures for the 12, 48, and 192-mcg. groups at the third harvest were 8.42, 8.14, and 10.05 mg., respectively. The corresponding figures at the fourth harvest were 2.46, 3.50, and 3.92 mg., respectively. The treated groups attained their maximal content of chlorophyll at the third harvest, as did the controls. The latter, however, contained up to 50% more chlorophyll *a* at that time than did the treated groups. At the final harvest the controls had about a three to fivefold greater amount of chlorophyll *a* than did the treated groups. It was apparent that chlorophyll synthesis progressed at about the same rate in treated and untreated groups during the first 2 weeks of the observation period. A study of the aforementioned data, however, indicates that catabolism of the pigment between the third and fourth-week harvests was significantly different in the treated groups. It was concluded that the significant reductions in the chlorophyll *a* content of the treated groups were probably due to a greatly increased rate of degradation of the pigment.

Considerable increases in the total sugar content of the 48 and 192-mcg. groups were found in the leaf-tops throughout the 4-week observation period, except at the second harvest. The increases at the third and fourth harvests were considered significant. It was noted that the sugar content in the leaf-tops of the controls decreased with age. A complete reversal of this trend was noted in the treated plants. Increased sugar content from GA treatment has been reported in *Digitalis purpurea* (25), *Digitalis lanata* (27), and buckwheat (28). Likewise, marked increases in the starch content of the leaf-tops of treated plants were noted from the second through the fourth weeks. Apparently GA stimulated carbohydrate production in the leaf-tops of stramonium. In a previous publication (6) it was suggested that GA induced changes in the carbohydrate and/or protein metabolism of stramonium. The results of this experiment indicate that GA increased the synthesis of carbohydrates in the leaf-tops and confirms our previous report (6) that the leaves of treated plants had a higher dry matter content than controls. This increased leaf density was attributed primarily to the increased carbohydrate content. It was doubtful that protein metabolism was directly affected. Brummett (23) has found generally no significant differences in the total, soluble, and protein nitrogen of the leaves of treated and untreated stramonium. However, significant decreases were noted in the nitrate nitrogen of the leaves of treated plants (23). These facts strongly suggest that the reduced concentration of alkaloids usually found in the aerial parts of stramonium treated with GA is due to an alteration in the normal metabolic pathways whereby alkaloids are synthesized by the plant. This might be explained in several ways. First, the increased carbohydrate was utilized for more extensive stem growth and synthesis of structural components of the plant. Second, the stimulation of carbohydrate production and subsequent storage diverted the utilization of carbohydrates, which might have been used for alkaloid synthesis, into other metabolic pathways. Either would result in the alteration of the usual pathways of alkaloid biogenesis normally existing in the plant.

The decreases of up to 57% in the petroleum-ether extract of the treated leaf-tops of stramonium suggest further that lipid biosynthesis was depressed. This solvent extracts volatile oils, fixed oils, and waxes. Stramonium seeds are rich in fixed oil. However, since the capsules and enclosed seeds were not weighed separately when leaf-tops weights were made, this experiment did not ascertain if the reduced lipid content was because of inhibition of lipid synthesis or a lower seed content in the treated plants. Further work is proceeding on this problem. Considerable decreases were noted in the content of the ether-soluble extractive of the leaf-tops at the first two harvests, while appreciable increases were found at the final harvest. Of greater interest was the apparent qualitative difference between the extracts found in the treated and untreated plants. The former were a yellowish-brown color, whereas the latter were dark green in color. A study is being continued to determine the difference in the composition of the ether-soluble extractive of the treated and untreated plants.

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Interaction of Pharmaceuticals with Schardinger Dextrins III

Interactions with Mono-Halogenated Benzoic Acids and Aminobenzoic Acids

By JOHN L. LACH and TING-FONG CHIN

A series of mono-halogenated benzoic acids and aminobenzoic acids were shown to undergo definite interactions with the cyclodextrins. The complexes formed are considered to be due, in part, to inclusion formation and to other attractive forces existing between the guest and host molecules. Data are presented to illustrate the effect of steric hindrance, polarity, resonance structure and inductive effect with respect to this interaction. Some stoichiometric data are also presented and several formation constants calculated.

THE CYCLIC STRUCTURE of the dextrins confers upon these compounds the ability to form mono-molecular inclusion compounds, where the guest is enclosed within the cyclodextrin void. In the case of these cyclodextrins there is just one host molecule formed by several constituents (glucose units) united through ordinary chemical bonds, which enclose one single molecule of the guest. This makes the cage of a permanent nature as opposed to clathrates (these may also have a cage-like hollow space in the center as in the dextrins) which are derived from crystalline lattices.

Generally speaking, formation of solid crystalline inclusion compounds can be explained on the basis that the molecules are held together by virtue of their spatial configuration, and these compounds are defined as chemically inert toward each other. True inclusion formation therefore implies that no attractive force or forces are of paramount importance between guest and host. However, in aqueous solution inclusion compounds of the cyclodextrins and various organic substances do exist (as reported from these laboratories) (1, 2), indicating that some attractive force or forces are operative which stabilize the guest molecule in the cage-like hollow space or the guest molecules are bonded to the cyclodextrin in some manner other than inclusion formation. The purpose of this investigation was to study this interaction further in terms of polarity, inductive effect, resonance structure, and steric hindrance with respect to the degree of interaction of these dextrins with various agents. A series of mono-halogenated and aminobenzoic acid derivatives were selected for this study.

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EXPERIMENTAL

Reagents:

α-Cyclodextrin $[\alpha]_D^{25}$ in water = +150.5 ± 0.5; β-cyclodextrin $[\alpha]_D^{25}$ in water = +162.5 ± 0.5;