(824×10^3 cells/ml.), and air flow (4.5 L. air/minute) within 8 days. An air flow rate of 27 L./minute did not increase growth materially.

Spearmint suspensions in the dual-carboy system completely hydrolyzed sucrose to dextrose and fructose by the third day of culture. Both dextrose and fructose were absent in the culture medium after 8 days.

None of six antifoam compounds studied appreciably affected spearmint suspensions. Dow Corning-B (500 p.p.m.) is suggested for foam control.

Five parts per million of either amphotericin, griseofulvin, and/or oxytetracycline inhibited spearmint suspension growth. Bacitracin (5 p.p.m.) is suggested for bacterial prophylaxis.

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Effect of Gibberellin and Other Treatments on the Germination and Subsequent Biogenesis of Alkaloids in *Datura stramonium* Linné

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Stramonium **seeds were subjected to two "cold treatments," to treatment with gibberellic acid (GA) and with concentrated sulfuric acid (SA). The rate and percentage of germination were markedly reduced by the cold treatments, whereas they were significantly increased by the GA and SA treatments. A threefold increase in germination percentage was noted in the former group and a twofold increase in the latter. Plants from the GA, SA, and control groups were allowed to grow to maturity. Characteristic gibberellin effects were not noted in plants receiving a gibberellin-seed treatment. However, the early growth of this group was markedly reduced. At the final harvest total plant weight was slightly less than controls. Fluctuations in the alkaloid concentration of the plant organs were noted. At the final harvest the total content of alkaloids was about 87 per cent of controls. The growth rate of the SA group was considerably lower than controls initially, but, the total dry weight at the final harvest approximated the controls. Both decreases and increases in the concentration of alkaloids in the plant organs were observed. The total plant alkaloids of this group at the final harvest were about the same as controls.**

Treatment of seeds with gibberellic acid (GA) has hastened germination and promoted an earlier and more uniform emergence of the seedlings of many crop plants (1–8) and several medicinal plants (9–11). Treatment of belladonna seeds with concentrated sulfuric acid resulted in an increase in the germination rate (12). Acid scarification and various types of "cold treatments" have been recommended as pregermination treatments of seeds to stimulate germination (13, 14). A review of the literature, however, has indicated that a study of such treatments on the subsequent growth and alkaloid biogenesis of stramonium has not been conducted.

The purpose of this study was (*a*) to determine and compare the effects on the germination of stramonium seeds of a freeze-thaw method (FT), a constant freeze method (CF), treatment with GA, and treatment with concentrated sulfuric acid

Received December 31, 1962, from the Department of Pharmacognosy, School of Pharmacy, Oregon State University, Corvallis.
Accepted for publication April 30, 1963.
Research Paper No. 451, Department of Pharmacognosy, School of Pharmacy, Oregon State University, Corvallis.
Presented to the Scientific Section, A. Ph. A., Washington, D. C., meeting, August 1960.
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(SA); and (*b*) to ascertain what changes such treatment would have on the subsequent growth and alkaloid biogenesis of the plant.

EXPERIMENTAL

Procedure—Five-hundred nearly uniform seeds were selected from a 1958 seed crop of *Datura stramonium*, var. *inermis* that had been gathered in March 1959 from a plant which had been allowed to over-winter. The seeds were divided into five equal groups. Group A was treated by alternate freezing and thawing during 1 week, *i.e.*, a 24-hour freezing period was followed by a similar thawing period. Group B was kept frozen for 1 week. Group C was treated with a 50 p.p.m. solution of GA[1] for 18 hours. Group D was treated for 1 minute with concentrated sulfuric acid and then washed free of the acid. Group E received no treatment and served as controls. The seeds were dusted with Fermocide[2] and planted on April 2, 1959, under greenhouse conditions in flats containing soil composed of sand, sandy loam, and peat moss. About 50 Gm. of complete organic fertilizer[3] was thoroughly mixed into each flat of soil prior to planting. Daily counts were made for a 5-week period, even though germination had apparently ceased by the end of the third week.

Since seeds of the control group (Group E) were not soaked in distilled water for 18 hours, but were planted directly in the soil in the dry state, a subsequent experiment was performed in 1963 to determine whether the beneficial response from the treatment with the GA solution was due to in i-bition of water or to a stimulating effect of the growth regulator. One-hundred seeds were soaked for 18 hours in a 50 p.p.m. solution of GA, and a similar number was soaked for the same period of time in distilled water. The time of planting, the experimental conditions, and observation period approximated that of the initial experiment.

On May 2, the second phase of this experiment was started. Twenty-four seedlings, each from the GA, SA, and control groups were transplanted into pots containing a mixture of sand, sandy loam, peat moss, and 50 Gm. of complete organic fertilizer. Since the plants grown from seeds receiving the cold treatments did not show a significant beneficial response during germination, they were discarded.

The plants from the three groups were arranged at random in the greenhouse. On May 19 (zero time), height measurements were taken and the first harvest of plants from each of the three groups was made. Height measurements were taken twice weekly thereafter, and the remaining plants were harvested in groups of eight from each of the test groups at the end of 2 and 4 weeks. The division of the plant into its morphological parts during each harvest, fresh and dry weight determinations, pulverization, pooling, and storage of the powdered material were conducted in a manner described in a previous publication (15). The pooled samples were analyzed for total alkaloids (calculated as scopolamine) by the Brummett-Sciuchetti method (16).

Germination Observations.—The seeds first germinated on the ninth day following planting. The seeds treated with GA germinated more rapidly, and a greater per cent of them germinated than did those from the other groups (Fig. 1). The seedlings from this group appeared healthy; they did not appear chlorotic; damping-off did not occur; and satisfactory growth was sustained throughout the 5-week germination period. About a threefold increase was noted in the germination percentage of the seeds treated with GA compared with untreated seeds. About twice as many plants germinated from seeds treated with sulfuric acid as from untreated seeds. The habit of this group approximated the controls. The FT and CF groups demonstrated lower rates and percentages of germination than the controls (Fig. 1). These seedlings, likewise, appeared to be similar in appearance to the controls.

The subsequent experiment which was performed to determine whether the favorable response from soaking seeds in a gibberellin solution was due to water imbibition or to a stimulus to germination by the GA solution, demonstrated a similar, although a less pronounced trend, as that described above. For example, the number of seedlings emerging from the GA-treated group was eight at the eighth day following planting, 18 at the 12th day, 25 at the 16th day, 35 at the 20th day, 40 at the 24th day, and 40 at the 28th day. The corresponding figures for the seeds soaked in distilled water only were 1, 7, 12, 15, 16, and 16. These results indicate that the increased rate of germination and higher percentage germination was due to the stimulating effect of the GA.

Growth Observations.—The plants were observed daily during the 4-week growth period following the first harvest. There were no appreciable differences

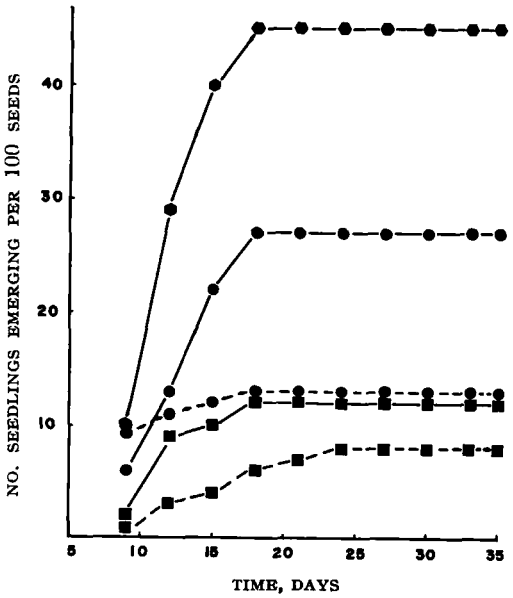

Fig. 1.—Effect of seed treatments on germination of *Datura*. Key: ●—●, gibberellic acid; ●—●, sulfuric acid; ● - - ●, controls; ■——■, freeze-thaw; ■ - - ■, constant freeze.

[1] The GA employed in this study was furnished through the courtesy of Dr. Edwin F. Alder, Agricultural Research Center, Eli Lilly and Co., Greenfield, Ind.

[2] Miller's Fermocide, 68% Ferbam, Miller Products Co., Portland, Oreg.

[3] Organic Morcrop, Chas. Lilly Co., Seattle, Wash. *Anal.*—5% total nitrogen, 3% available phosphate, 2% available potash.

TABLE I.—AVERAGE HEIGHTS[a] OF STRAMONIUM PLANTS

Treatment	Harvest Time, Wks.	Av. Height, cm.	Control, %
Control	0	11.04	...
SA	0	10.35	93
GA	0	11.16	101
Control	2	28.59	...
SA	2	28.63	100
GA	2	26.61	93
Control	4	48.63	...
SA	4	50.95	104
GA	4	44.36	91

[a] Heights in cm. are the average of eight plants.

in the habit of the treated and untreated groups. All plants appeared healthy and vigorous and none were chlorotic. Only slight changes in height were noted in the treated groups (Table I). At the final harvest, the SA group was slightly taller than controls, while the GA group was shorter. Marked stem elongation was not observed in the latter.

Fresh and Dry Weights.—Appreciable reductions in the dry weights of plants grown from treated seeds were generally noted at the first two harvests (Table II). At the terminal harvest the total dry weight of the SA group was about 96% of the controls, and that for the GA group was about 91%. These decreases were not considered significant. The growth rate of the SA group, although less than controls, was generally somewhat higher than the GA group. A study of the dry weight data indicated that the seed treatments retarded growth initially, but these plants approached the controls by the final harvest.

Concentration of Alkaloids.—The alkaloid analysis was performed on pooled samples by the Brummett-Sciuchetti method (16). Considerable fluctuations were noted in the concentration of alkaloids in the organs of the plants grown from treated seeds (Table III). Generally, slightly reduced concentrations compared with controls were noted in the leaves-tops of the treated groups. The stems of the plants grown from seed treated with sulfuric acid had an appreciably lower concentration of alkaloids at the first harvest, but a slight increase at the final harvest. A reverse trend was noted in the GA group. The roots of the SA group had a significant increase (147% of controls) at the second- and fourth-week harvests. On the other hand, the roots of plants grown from seeds treated with GA had a significant reduction at zero time but only a slight decrease at the fourth week. The SA treatment of seeds elicited a favorable response more often than did the GA treatment.

Total Plant Alkaloids.—The total alkaloids per plant and per plant organ were obtained by multiplying the dry weight of the plant part by the per cent of alkaloids obtained from the alkaloid analyses and expressing the results in milligrams (Table IV). It was noted that at each harvest plants grown from treated seeds produced less total alkaloids than the controls. The most significant decrease was at the second harvest where the total alkaloid content per plant of the GA group was only 64% of the controls. This was because of a significant decrease in plant growth and a marked reduction in the concentration of leaves-tops alkaloid. By the final harvest this group had 87% of the total alkaloids of the controls. On the other hand, the total alkaloids per plant of the SA group showed an appreciable decrease compared with controls at the first harvest, a considerable reduction at the second harvest, and approximately the same content at the final harvest. In general, the aerial parts of the treated groups showed significant decreases in total alkaloids at the first harvest, marked reductions at the second harvest, and only minor decreases

TABLE II.—WEIGHTS OF STRAMONIUM PLANT PARTS (AV./PLANT/GROUP)

Treatment and Harvest Time, Wks.	Total Wt.			Leaves-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, 0	3.44	0.42	...	1.71	0.23	...	0.89	0.10	...	0.84	0.09	..
SA, 0	3.53	0.37	88	1.61	0.19	83	0.80	0.07	70	1.12	0.11	122
GA, 0	3.02	0.29	69	1.57	0.16	70	0.52	0.06	60	0.93	0.07	78
Control, 2	36.58	3.49	...	22.77	2.39	...	10.50	0.78	...	3.31	0.32	...
SA, 2	29.06	2.68	76	17.61	1.86	78	8.48	0.56	72	2.97	0.26	81
GA, 2	27.09	2.60	74	16.17	1.77	74	7.31	0.56	72	3.07	0.27	84
Control, 4	82.15	11.69	...	49.12	6.80	...	23.00	3.62	...	10.03	1.27	...
SA, 4	79.90	11.25	96	49.67	6.66	98	21.50	3.55	98	8.73	1.04	82
GA, 4	77.41	10.72	91	47.10	6.52	96	22.05	3.22	89	8.26	0.98	77

TABLE III.—CONCENTRATION OF ALKALOIDS[a] IN STRAMONIUM PLANT PARTS

Treatment and Harvest Time, Wks.	Leaves-Tops		Stems		Roots	
	mg./Gm.	Control, %	mg./Gm.	Control, %	mg./Gm.	Control, %
Control, 0	4.1	...	4.3	...	2.9	...
SA, 0	3.8	93	3.2	74	2.8	97
GA, 0	4.4	107	4.6	107	1.8	62
Control, 2	3.9	...	2.8	...	0.95	...
SA, 2	4.3	110	2.8	100	1.4	147
GA, 2	3.2	82	3.0	107	0.95	100
Control, 4	5.3	...	2.2	...	1.9	...
SA, 4	5.2	98	2.4	109	2.8	147
GA, 4	5.0	94	1.9	86	1.7	89

[a] Total alkaloids were calculated as scopolamine.

TABLE IV.—TOTAL ALKALOID CONTENT[a] (MG.) OF STRAMONIUM

Treatment and Harvest Time, Wks.	Per Plant		Leaves-Tops		Stems		Roots	
	Total Alkaloids	Controls, %	Total Alkaloids	Controls, %	Total Alkaloids	Controls, %	Total Alkaloids	Controls, %
Control, 0	1.63	...	0.94	...	0.43	...	0.26	...
SA, 0	1.25	77	0.72	77	0.22	51	0.31	119
GA, 0	1.11	68	0.70	75	0.28	65	0.13	50
Control, 2	11.80	...	9.32	...	2.18	...	0.30	...
SA, 2	9.93	84	8.00	86	1.57	72	0.36	120
GA, 2	7.60	64	5.66	61	1.68	77	0.26	87
Control, 4	46.41	...	36.04	...	7.96	...	2.41	...
SA, 4	46.06	99	34.63	96	8.52	107	2.91	121
GA, 4	40.39	87	32.60	90	6.12	77	1.67	70

[a] Calculated from dry weight and alkaloid analyses data; per plant = leaves-tops + stems + roots.

at the terminal harvest. The roots of the SA group contained considerably more alkaloids than controls throughout the 4-week growth period, while the GA group had appreciably less total alkaloids (Table IV).

DISCUSSION AND CONCLUSIONS

The GA-treated seeds showed about a threefold increase over untreated seeds in germination percentage and also demonstrated a more rapid rate of germination. Treatment of belladonna seeds (10) and of hyoscyamus seeds (9) with GA has also promoted an earlier and more uniform emergence of seedlings and caused a higher percentage of seeds to germinate. Throughout the observation period the habit of these plants closely resembled the controls, except that they were slightly shorter than plants grown from untreated seed. Characteristic gibberellin effects, such as taller and spindlier plants, greater internodal elongation and a chlorotic appearance, have been reported (11,15–19) when plants were treated with GA. These effects were not observed in plants grown from seed treated with GA. It was noted from dry weight data that during the early stages of growth the seed treatment induced a deleterious effect on growth. Although these plants weighed less than controls at the final harvest the reduction in plant growth was not considered significant. This is in contrast to a generally beneficial effect usually reported for *Daturas* treated with GA (11, 15, 16, 18).

Inconsistent trends were observed regarding the concentration of alkaloids in the plant organs. Both increases and decreases, compared with the control group, were noted at the various harvests. The increased concentration in the aerial parts of the plant noted in some cases from treatment of seeds with GA differs from the reductions usually noted when *Daturas* are treated with the growth hormone (15, 16, 18). Although the total alkaloid content of the GA group was somewhat less than controls at the final harvest, the seed treatment with GA was considered beneficial since a greater and more uniform emergence of seedlings was obtained. The results obtained by the gibberellin-seed treatment in this experiment were generally similar to that in the study with belladonna seeds (10) which was being conducted concurrently with this one. However, a general increase in alkaloid formation was noted in belladonna grown from gibberellin-treated seeds.

Treatment of seeds with sulfuric acid induced about a twofold increase in germination percentage over untreated seeds. A more rapid and uniform emergence of seedlings was also noted in this group. The habit of this group approximated the controls. The growth rate of the SA group was considerably slower than the controls initially, however, at the final harvest the total dry weight of this group was about the same as controls. Both decreases and increases were noted in the concentration of alkaloids in the plant organs throughout the 4-week growth period. No significant difference in total plant alkaloids was noted in the SA group compared with controls at the final harvest. This differed from belladonna grown from seed treated with sulfuric acid since belladonna was stunted in growth and contained about 40% of the alkaloids of controls (10).

The application of various cold treatments to stramonium seeds did not cause an increase in germination. Actually the germination rate and total germination were considerably less in seeds treated by the constant freeze method and the freeze-thaw method. It was concluded that treating stramonium with gibberellic acid or sulfuric acid was beneficial since both the rate of germination and the number of seedlings germinating were significantly increased. The growth and total alkaloid content of the treated groups were not materially different from controls at the final harvest. These treatments are suggested when it is desirable to increase the germination of stramonium, especially when seeds are planted in late winter or early spring.

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