our 50 seconds^{-1}, the choice in this work being a practical working range for extrapolation on the log stress-log shear rate plot for the rheometer used.

In the judgment of relative merits of different suspending systems, two ratios can prove valuable for comparison. These are the ratios of the suspendability to the pourability index and the reciprocal of this ratio. The first is a measure of suspendability of a given degree of flow, the other the ease of pouring for a definite degree of suspending ability. The first ratio defines the relative ordinate value in Fig. 8 for a given abscissa and the reciprocal the abscissa for a given value of the ordinate.

Both are useful terms to use to characterize in tabular form the data of Fig. 8 for given values of pourability or suspendability. The ratio, its parameters, along with the critical concentration to give a suspendability of unity, permit an excellent system characterization as is seen in Table IV.

SUMMARY

The use of log shear stress-log shear rate for low shear rate data is suggested as desirable for determining whether yield value character is, or is not, implicit in a given rheogram.

The practical difficulty of extrapolation to a yield value may, in practice, be estimated by either a Casson or Fitch plot.

Many gum systems, on increase in concentration of gum, show the development of yield value characteristics. These characteristics may be markedly enhanced by a second agent whose own yield value will be developed at low concentration.

Since yield value is a direct measure of suspendability and viscosity of pourability, a suspendability-pourability relationship has been developed for a series of gum systems. Tables and plots suitable for suspending gum characterization are given.

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Action and Interaction of Gibberellic Acid and B995 on *Datura innoxia*

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Thirty-three-day-old *Datura innoxia* seedlings were treated weekly for a period of 4 weeks with 50 mcg. of GA, 100 to 1000 p.p.m. solutions of B995, or combinations of both. The GA-treated plants indicated significantly increased height, increased stem dry weight, and generally decreased alkaloid concentration. Plants treated with the combination of the growth factors closely paralleled those treated with GA. Plants treated with B995 closely resembled controls, except for a decrease in alkaloid concentration in the roots at the last harvest. The results of a modified Dragendorff analysis are reported.

RESEARCH recently completed on several different compounds revealed that B995, known chemically as *N*-dimethylaminosuccinamic acid, produced growth retardation in plants (1). Numerous chemical compounds having the specific effect of retarding stem elongation have recently been described (2–9). These growth retardants cause a marked decrease in stem and petiole elongation and exert relatively little influence on root development or leaf expansion, unless administered in high doses. Lockhart (7) has indicated that several of the growth retardants interact competitively with gibberellin on stem growth, and that they act to retard stem elongation by partially blocking the system which provides active gibberellin to the growth mechanism. Others have indicated apparent interactions between gibberellin and certain retardants in *Ulothrix* growth (10), cell division of chrysanthemum (11), and in bean internode growth (12).

Most growth inhibitors are known to stimulate growth at dilute concentrations and inhibit at high concentrations (4, 10, 13). Low concentrations of Amo-1618 stimulated the growth of cucumber seedlings, whereas higher concentra-

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tions retarded growth (4). A similar effect was noted when onion roots were treated with maleic hydrazide (13) and when *U. subtillisma* was treated with AMAB (10).

The following general gibberellin effects have been reported in the solanaceous plants producing tropane alkaloids: increased internodal elongation, taller and spindlier plants, slightly chlorotic leaves, increased stem growth, and reduction in the concentration of alkaloids in the aerial parts (14–20).

The purpose of this investigation was (*a*) to determine whether B995 would retard the growth and/or influence the biogenesis of alkaloids and other components in *Datura innoxia;* (*b*) to ascertain whether this plant responded to gibberellic acid (GA) as did other solanaceous plants; and (*c*) to determine whether interaction occurred when the plant was treated with a combination of growth chemicals which generally induce opposite effects on stem growth.

EXPERIMENTAL

Procedure.—Ninety-six *D. innoxia* seedlings which were approximately 33 days old were used in this study. The seedlings were grown from a 1961 seed crop at Oregon State University and were kept under greenhouse conditions at a temperature ranging from 65 to 85° F. throughout the experiment. The seedlings were transplanted to individual 1-gal. metal cans 9 days before the experimental period began and were randomized on a greenhouse bench. The soil mixture consisted of one part sand, two parts loam, and 5 Gm. of Organic Morcrop[1] fertilizer per can.

The experimental period started on July 5, 1962 (zero time), and lasted 28 days. Three harvests were made, at 0, 2, and 4 weeks. Each harvest consisted of eight plants from each of the four groups, *viz.*, control plants, GA-treated plants, B995-treated plants, and plants receiving a combined treatment. The first harvest, at zero time, however, consisted of 32 plants, which were considered controls since none had been treated. Weekly thereafter, the following treatments were instituted: the GA-treated group received 50 mcg. of GA in a volume of 0.02 ml., which was applied with a micropipet onto the growing apex of the plant. The B995 group received increasing concentrations of the chemical, since visible growth retardation was not observed initially with the lower concentration of the growth inhibitor. At zero time, a 100 p.p.m. solution of B995[2] was used. The next week a 500 p.p.m. solution was employed. The last two treatments consisted of 1000 p.p.m. solutions. The chemical was dissolved in distilled water, and two drops of Triton X100, a nonionic emulsifier, was added to each liter of solution to provide better spreading properties. The treatment was a spray to "run-off." Specially prepared paper shields prevented the solutions from entering the soil. Plants receiving the combined treatment were administered the gibberellic acid 2 days before treatment with B995. The latter was administered weekly in the same concentration as that used on the plants receiving it alone.

At harvest time, each plant was immediately washed, cleaned, and the moisture was removed with towels. Then the plant was divided into leaf-tops, roots, and stem portions. The leaf-tops and stems were cut into small segments, and the fresh weight of each portion was taken immediately. Dry weights were taken after drying 48 hours in a forced air drier at 48.5° C. The plant parts were then ground to a No. 40 powder in a Wiley mill, pooled according to treatment and harvest, and stored in colored glass containers in a desiccator until subsequent analyses were performed.

Growth Effects.—The GA and combination-treated plants grew more rapidly and matured faster than the others. More and larger lateral buds and shoots were noted on these plants than in the controls and B995-treated plants. The stems of the latter were not so woody, nor did these plants bloom so fast as the GA and combination-treated plants.

Height measurements, taken twice weekly, showed that there was no difference between GA and combination-treated plants, which grew significantly taller and more rapidly than controls and the B995-treated plants (Fig. 1). Throughout the experiment, the growth pattern of the controls and B995-treated plants were similar. At the end of the experimental period, both GA and combination-treated plants had attained heights about 146% of controls, whereas B995-treated plants were about 10% shorter than controls (Fig. 1).

Fresh and Dry Weights.—The total dry weight of the GA-treated group was about 15% greater than control plants at the second and fourth weeks. This increase was due to about a 60% gain in stem weight (Table I). The leaf-tops weight of this

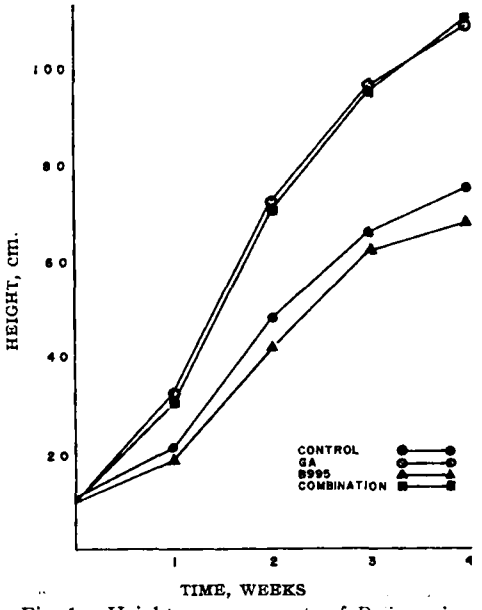

Fig. 1.—Height measurements of *D. innoxia.*

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

[2] The B995 employed in this study was furnished through the courtesy of Dr. J. A. Riddell, Plant Physiologist, Naugutuck Chemical, Betheny, Conn.

TABLE I.—WEIGHTS OF *Datura* PLANT PARTS[a]

Treatment and Harvest Time, Wk.	Total Wt. Fresh, Gm.	Total Wt. Dry, Gm.	Total Wt. Control Dry Wt., %	Leaf-Tops Fresh, Gm.	Leaf-Tops Dry, Gm.	Leaf-Tops Control Dry Wt., %	Stems Fresh, Gm.	Stems Dry, Gm.	Stems Control Dry Wt., %	Roots Fresh, Gm.	Roots Dry, Gm.	Roots Control Dry Wt., %
Control, 0[b]	4.18	0.276	...	3.11	0.223	...	0.57	0.028	...	0.50	0.025	...
Control, 2	55.92	5.43	...	32.62	3.38	...	14.72	1.35	...	8.58	0.70	...
GA, 2	63.46	6.18	114	27.87	3.39	100	29.41	2.21	163	6.18	0.58	83
B995, 2	53.93	5.58	103	32.12	3.71	109	13.88	1.19	88	7.93	0.68	97
Combination, 2	60.59	5.40	99	27.31	3.20	92	26.98	1.16	123	6.30	0.54	77
Control, 4	118.90	18.99	...	47.50	7.64	...	39.70	6.99	...	31.70	4.36	...
GA, 4	118.10	21.80	115	46.40	8.08	106	56.40	11.13	160	15.30	2.59	59
B995, 4	116.60	18.75	99	50.90	7.52	98	36.40	6.22	89	29.30	5.01	115
Combination, 4	109.10	19.41	102	40.90	7.08	93	54.20	10.02	143	14.00	2.31	53

[a] Mean weights per group of eight plants. [b] Mean weights of all four groups which were harvested but not treated at 0 time.

group gained slightly, whereas the root weight was markedly reduced. The decrease of 41% in the dry weight of the roots at the fourth week was considered significant. In general, the total dry weight and the dry weights of the individual organs of the B995 group approximated that of the controls. Although the total dry weight of the group receiving the combined treatment was about the same as the controls, significant differences were found in stem and root dry weights (Table I). Stem dry weights increased 23 and 43% at the second and fourth weeks, respectively. The increased stem weight of this group was not so great as that of the GA-treated group. Root dry weights were reduced 23 and 47% in the combination group at the second and fourth weeks, respectively. These decreases were larger than that noted in the group treated with GA.

Analysis for Alkaloids.—The dried plant parts, using pooled samples, were assayed for alkaloid concentration, expressed as scopolamine, according to the Brummett-Sciuchetti method (16). Two extractions were made per group, and each group was analyzed in duplicate. When duplicate determinations did not agree, two further extractions were made for each group.

The concentration of alkaloids (mg./Gm.) in the organs of the GA-treated plants was markedly reduced, except that a gain was noted in the leaves of this group at the terminal harvest (Table II). The B995-treated plants at the final harvest indicated about a 17% increase in the leaves and a 35% decrease in the roots, with no appreciable change in the stems. All organs of this group contained less alkaloid at the second week harvest. Significant decreases were noted in all organs of the plants receiving a combination treatment at the second and fourth week harvests (Table II). The reductions in the plant organs of this group were greater than either of the other two groups receiving treatments. In general, the gibberellin effect appeared to be potentiated in plants receiving the combination 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. GA-treatment induced a slight decrease in the total alkaloids per plant (Table III). However, the accumulation of alkaloids in the leaf-tops was increased, whereas total root alkaloids were significantly reduced. The B995 group approximated the controls in total alkaloids per plant. However, appreciable increases were noted in leaf and root alkaloids at the final harvest. In general, significant reductions per plant and per plant organ were noted in the total alkaloids of plants receiving the combination treatment.

Selective Solvent Extraction.—Duplicate 2-Gm. samples of powdered leaf material from each group were selectively extracted in a Soxhlet apparatus in sequence with the following solvents: petroleum ether U.S.P., anhydrous ether C.P., ethyl alcohol U.S.P., distilled water, sodium hydroxide solution (0.2%), and 1% hydrochloric acid according to a modified Dragendorff method (21). The powdered material was subjected to each solvent until completely extracted. Second duplicate determinations were carried out when agreement was not obtained between two samples from the same group. The weights of the dry extracts, obtained

TABLE II.—CONCENTRATION OF ALKALOIDS[a] IN *Datura* PLANT PARTS

Treatment and Harvest Time, Wk.	Leaf-Tops Alkaloids, mg./Gm.	Leaf-Tops Controls, %	Stems Alkaloids, mg./Gm.	Stems Controls, %	Roots Alkaloids, mg./Gm.	Roots Controls, %
Control, 0	3.07	...	8.50	...	5.01	...
Control, 2	4.44	...	5.77	...	5.50	...
GA, 2	4.04	91	3.79	66	4.86	88
B995, 2	4.40	99	4.88	85	4.80	87
Combination, 2	2.98	67	3.31	57	3.34	61
Control, 4	2.56	...	3.49	...	6.12	...
GA, 4	3.24	126	2.28	65	4.00	65
B995, 4	2.99	117	3.68	105	4.00	65
Combination, 4	1.50	59	2.18	62	4.68	76

[a] Expressed as scopolamine.

TABLE III.—TOTAL ALKALOID CONTENT[a] (mg.) OF *D. innoxia*

Treatment and Harvest Time, Wk.	Per Plant		Leaf-Tops		Stems		Roots	
	Total Alkaloids	Controls, %	Totals Alkaloids	Controls, %	Total Alkaloids	Controls, %	Total Alkaloids	Controls, %
Control, 0	1.17	...	0.80	...	0.25	...	0.12	...
Control, 2	25.65	...	15.01	...	6.79	...	3.85	...
GA, 2	24.88	91	13.70	91	8.36	123	2.82	73
B995, 2	25.39	99	16.32	109	5.81	86	3.26	85
Combination, 2	16.83	66	9.54	64	5.49	81	1.80	47
Control, 4	65.96	...	18.79	...	25.79	...	21.38	...
GA, 4	61.92	94	26.18	139	25.38	98	10.36	48
B995, 4	70.42	107	22.48	119	22.89	89	25.05	112
Combination, 4	43.27	66	10.62	56	21.84	85	10.81	51

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

by evaporation of the solvent on a water bath, followed by oven-drying at 48.5° C. for 24 hours, are given in Table IV.

Considerable changes in the concentrations of the various extractives of the leaf-tops were noted in the treated groups. Considering the plants gathered at the final harvest as indicative of specific effects from the treatments, the following differences from controls were noted. Decreases of 17, 26, and 31% were found in the petroleum ether extract of the GA, B995, and combination groups, respectively. This suggests that lipid synthesis and/or accumulation was reduced in the leaf-tops. The ether extractive of the GA group only was affected (a 25% decrease). About a 14% reduction was observed in the alcohol-soluble extractive of the B995 and combination groups, whereas a slight increase was noted in the GA group. Reductions of about 22% were noted in the water extracts of the GA and combination groups; an 11% decrease was found in the B995 group. The sodium hydroxide extract was increased about 14% in the GA group, while decreases of 28% were noted in the B995 and combination extracts, respectively. No appreciable changes were found in the hydrochloric acid extracts. Where appreciable changes occurred in the various fractions, the B995 and combination groups indicated decreases from controls. The reductions were of a greater magnitude in the group receiving a combined GA and B995 treatment than in the group treated with B995 alone.

RESULTS AND DISCUSSION

Slight growth retardation was induced by B995. This group of plants was about 90% as tall as the controls. Although some fluctuations were noted in the dry weights of the various organs of the B995 group, the general growth pattern was similar to the controls. Inconsistent trends were noted regarding alkaloid concentration. All of the organs of the plants treated with B995 had a lower alkaloid concentration at the second week, while at the fourth week a 17% increase was found in the leaf-tops and a decrease in the roots. The total alkaloid content per treated plant approximated the controls. At the final harvest appreciable decreases were noted in the petroleum ether, alcohol, and sodium hydroxide extracts. This indicates that treatment with B995 did markedly affect the quantity of components soluble in these solvents. It is the authors' opinion that more striking effects might have been induced on internode elongation (height) had a much higher concentration of B995 been employed during the treatment period.

D. innoxia responded to GA-treatment, regarding growth and concentration of alkaloids in the aerial parts, in a manner similar to that previously reported for *D. stramonium* (14–16, 19) and *D. meteloides* (18). The general trends in growth and effect on alkaloid concentration as reported by Kapoor and Kaul (20) in *D. innoxia* were confirmed. However, the concentration of alkaloids in the leaf-tops and roots of our plants was about twice as high in both treated and untreated groups. This difference was most likely due to divergences in the environmental conditions and the experimental design of the two investigations. In addition to a favorable effect on growth (as indicated by increased dry weights) the GA-treated plants flowered earlier than controls. The stems also appeared to be more woody. The total alkaloid content per plant at the final harvest was about 6% less than controls. This was due to significant reductions in the concentration of alkaloids in the stems and roots accompanied by a significant increase in leaf-tops alkaloids. About a 20% decrease was noted at the final harvest in the petroleum ether, ether, and water-soluble extractives of the leaf-tops of the GA group. Slight increases were noted in the alcohol-soluble and sodium hydroxide-soluble extractives. The increase in the former correlates with the increased concentration of alkaloids found in the leaf-tops of the GA group.

TABLE IV.—RESULTS[a] OF SELECTIVE SOLVENT EXTRACTION OF LEAF-TOPS OF *D. innoxia*

Treatment and Harvest Time, Wk.	Petroleum Ether	Ether	Alcohol	Water	NaOH	HCl
Control, 2	34.8	15.3	213.4	101.7	142.2	88.5
GA, 2	36.1	13.4	245.6	109.3	151.0	89.8
B995, 2	34.6	17.0	214.0	115.6	147.7	92.5
Combination, 2	33.0	12.0	266.5	144.5	190.1	106.5
Control, 4	26.4	11.5	367.1	158.9	305.9	85.2
GA, 4	21.9	8.6	390.4	123.9	348.8	81.3
B995, 4	19.5	11.9	316.1	140.9	217.7	82.2
Combination, 4	18.3	10.4	319.8	123.8	210.9	76.9

[a] Concentrations given in mg./Gm.

A most interesting aspect of this experiment was the apparent synergistic effect treatment with B995 had when it was combined with GA. The gibberellin effects (on internode elongation and on alkaloid concentration) generally appeared to be potentiated. Plants treated with B995 only were shorter than controls. GA-treatment induced about a 46% increase in height. Plants receiving the combined treatment were also approximately 46% taller than controls. In this case, as well as with the decrease in root dry weight, the combination treatment apparently potentiated the effect noted with GA alone. The stem alkaloid concentration at the last harvest was about 65% of controls in the GA group, 105% in the B995 group, and 62% in the group receiving the combined treatment. In general, when reduced concentrations of alkaloids were found in the organs of the GA group, the decreases were of a greater magnitude in the combined group. The total alkaloid content per plant among the treated groups was the greatest in the B995 group, considerably less in the GA group, and the least in the combined group. Where appreciable changes were noted in the various selective solvent extracts of the B995 group, the variations were greater when B995 was combined with GA than when B995 was employed alone.

CONCLUSIONS

B995 had a slight effect on the retardation of stem elongation at the concentrations employed. The total alkaloid content of this group approximated the controls. However, results from the selective solvent extraction procedure indicated marked decreases in the quantity of the ether, alcohol, and sodium hydroxide extracts from the leaf-tops.

This plant responded to GA-treatment in a manner similar to other solanaceous plants. The following characteristic gibberellin effects were noted: taller and spindlier plants, significantly increased internodal elongation, slightly chlorotic leaves, woodier stems, a significant increase in stem weight, and a general reduction in the concentration of alkaloids in the aerial parts of the plant. Also, decreases of about 20% were found in the petroleum ether, ether, and water-soluble extractives of the the leaf-tops.

A most interesting interaction occurred when the plants were treated with a combination of growth chemicals. Only slight growth retardation, as indicated by height, was noted in *D. innoxia* treated with B995. Significantly increased height was induced with GA. However, the gibberellin effect predominated or was apparently potentiated when both treatments were employed. A similar trend was usually noted in the alkaloid concentrations of the various plant organs. This suggests that the concentration of B995 used in this experiment was not sufficiently high to induce definite growth retardation, but was low enough to demonstrate a slight stimulatory (gibberellin) effect. Others (4, 10, 13) have shown that growth inhibitors at dilute concentrations stimulate growth, whereas at high concentrations growth is inhibited.

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