Anal.[4]—Calcd. for $C_{15}H_{30}N_3O_3P$: C, 54.38; H, 9.06; N, 12.69. Found: C, 54.23; H, 9.19; N, 12.85.

Infrared absorption bands $\lambda_{max.}^{CHCl_3}$ (μ): 2.93 (w) (N—H); 3.35 (s) (C—H); 5.78 (s) (C=O); 6.95 (s) (sh at 6.85) (C—CH_3; C—CH_2); 7.28 (s) (C—CH_3); 7.65 (m) (P=O); 8.1–8.55 (s) (C—O; C—N); 8.84 (s) [C(CH_3)$_2$]; 9.35 (s); 10.38 (s) 10.55 (w); 11.10 (w).

The NMR spectrum (in $CDCl_3$, with tetramethylsilane as the internal standard) shows, at δ = 1.35 p.p.m., a singlet for the 24 methyl protons of the aziridine groups. In comparison, the urethan signals (δ = 4.10 and 1.30 p.p.m.) are very small and blurred.

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Effects of Amo-1618, Maleic Hydrazide, and Gibberellin Seed Treatment on the First and Second Generation of *Datura tatula*

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Treatment of *Datura tatula* with Amo-1618 increased the fresh and dry weights of the first-year plants, but decreased the alkaloid content. Maleic hydrazide (MH) treatment induced an increase in plant weight and total alkaloid content but a decrease in plant height. Second generation studies indicated a reduction of the fresh and dry weights of the Amo-1618 group, a considerable decrease in the alkaloid concentration of the MH group, and a decrease in the total alkaloid content of both groups. The chlorophyll concentration was not affected. A selective solvent extraction was performed on the leaf-tops. Gibberellin treatment of seeds obtained from plants previously treated with the chemicals generally reversed any inhibitory effect demonstrated by the chemicals on growth, alkaloid concentration and content, and the concentration of various selective-solvent fractions. Gibberellin seed treatment of the controls caused decreases in fresh weight, total alkaloid content, and the alcohol-soluble fractions of the plant.

AMO-1618 (4-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate) in appropriate concentrations inhibits stem elongation of plants (1–6). It is classified as a growth retardant, *i.e.*, a chemical that slows cell division and cell elongation in shoot tissues and regulates plant height physiologically without formative effects (1). Wirwillie and Mitchell (7) found that Amo-1618 was translocated into the seeds of treated black valentine snap beans, and growth retardation was noted in the following generation of plants. Mutual antagonism has been found between gibberellin and Amo-1618, or similar acting growth retardants, in altering plant growth. This antagonism has been reported for the stem growth of bean (8, 9) and potato (10) and in the cell division of chrysanthemum (11). Lockhart (9) indicates that several growth retardants interact competitively with gibberellin on stem growth, and they act to retard stem elongation by partially blocking the system which provides active gibberellin to the growth mechanism. Zeevaart and Lang (12) have shown that gibberellin completely overcomes the inhibition of flower formation induced by Amo-1618 in *Bryophyllum daigremontianum*. Kende *et al.* (13) found that gibberellic acid (GA) biosynthesis, but not growth, was inhibited

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by Amo-1618 in *Fusarium moniliforme*. Bennett and Sciuchetti (14) sprayed *Datura meteloides* with a 1000 p.p.m. solution of Amo-1618 and found that the root alkaloid content was increased, while the concentration of leaf chlorophyll was decreased. No significant effects were noted in plant height or weight.

Maleic hydrazide (MH), 1,2-dihydro-3,6-pyridazinedione, may have an antiauxin effect (15) and is considered to be a growth inhibitor rather than a growth retardant (1, 16, 17). Brian and Hemming (18), working with a variety of peas not responding to GA, concluded that GA did not reverse MH-induced inhibition of stem growth but that it probably interfered with the normal growth response at some stage before GA exerts its effect. Bukovac and Wittwer (19) reported that GA overcame the inhibitory effects of MH on the epicotyl growth of beans. Kato (20) found that MH-induced inhibition of shoot growth in cucumber seedlings was partly prevented by GA. From these conflicting reports, it is difficult to assess the true status of MH–GA interactions on plant growth.

GA treatment of belladonna seeds did not induce significant changes in plant height or weight but caused a slight reduction in the total alkaloid content (21). A similar trend was noted with *Datura stramonium*, var. *inermis* (22).

The purpose of this study was (*a*) to determine the effects of Amo-1618 and MH on the growth and alkaloid production of *Datura tatula* Linné, (*b*) to ascertain whether the effects induced by these chemicals would persist in the second-generation plants, and (*c*) to observe whether GA treatment of seeds obtained from plants previously treated with these chemicals would reverse any inhibitory effects which might be noted in the second-generation plants.

EXPERIMENTAL

Procedure.—The *D. tatula* plants employed in this study were grown under greenhouse conditions. Seeds were obtained from plants cultivated in the Oregon State University drug garden in 1958. The germination of the seeds, transplantation of the seedlings into individual 1-gal. metal containers, and soil composition are described in a previous publication (23). Twenty-seven uniform seedlings were selected and divided into three groups of nine each according to the following plan: untreated plants (controls), Amo-1618-treated plants, and MH-treated plants. The labeled plants then were randomized on the greenhouse bench.

On April 26, 1960 (zero time), treatment was instituted. The chemicals[1] were administered in the form of a spray to the youngest leaves of the plant. Three sprays of approximately 0.1 ml. each of a 100 p.p.m. aqueous solution of the specific chemical were applied to each plant of each treatment group. The MH group was treated once weekly—the Amo-1618 group, twice weekly. Specially prepared paper shields prevented the solutions from entering the soil. The treatment schedule, observation of the plants, and twice-weekly height measurements were conducted from zero time until June 6. At that time, six of the nine plants from each group were harvested. The plants were 53 days old at harvest time. The remaining three plants from each group were allowed to mature to provide seeds for the second generation study. The division of the plant into its morphological parts at harvest time, fresh and dry weight determinations, pulverization, and storage of the powdered materials were conducted in a manner described in a previous publication (23).

The second-generation studies were performed in 1962. The seeds were obtained from the 1960 study group previously described. These seeds were pooled according to group (controls, Amo-1618, and MH). Two-hundred seeds from each group were divided into two subgroups of 100 each. One subgroup was soaked in a vial containing distilled water; the other subgroup was soaked in a glass vial containing 30 ml. of a 50 p.p.m. solution of GA.[2] The seeds were allowed to soak for 48 hr. This procedure provided the following six series of plants: series Amo-1618, plants grown from seeds of plants treated previously with Amo-1618; series AGA, plants grown from seeds of plants treated previously with Amo-1618 and whose seeds were treated further with GA; series MH, plants grown from seeds of plants treated previously with MH; series MGA, plants grown from seeds of plants treated previously with MH and whose seeds were treated additionally with GA; series C (controls), plants grown from seeds of untreated plants; series CGA, control seeds which also received a GA treatment.

Subsequently, 10 uniform seedlings from each series were transplanted 3 days prior to zero time into 1-gal. metal cans filled with a soil mixture similar to that used in the first generation study. The transplanted seedlings were then randomized on a greenhouse bench. On August 13 (zero time), twice weekly height measurements were commenced, and the plants were examined periodically for any morphological changes during the ensuing 1-month observation period. Harvesting of the 67-day-old plants included division of the plant parts into leaftops, stems, roots, and capsule portions. Fresh and dry weight determinations, pulverization, and storage of the powdered material were carried out in a manner similar to that for the first generation plants.

Analysis for Alkaloids.—The alkaloid concentration, calculated as scopolamine, was determined by the Brummett-Sciuchetti method (24). A minimum of three duplicate determinations was carried out on each sample of pooled material.

Total Plant Alkaloids.—The total alkaloid content per plant and per plant organ was obtained by multiplying the dry weight of the plant part by

[1] The Amo-1618 was prepared by dilution of Plant Tranquilizer, Rainbow Color and Chemical Corp., Northridge, Calif. The maleic hydrazide was obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio.

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

TABLE I.—MEAN WEIGHTS OF DATURA PLANT PARTS[a]

Treatment	Total Wt. — Fresh, Gm.	Dry, Gm.	Leaf-Tops — Fresh, Gm.	Dry, Gm.	Stems — Fresh, Gm.	Dry, Gm.	Roots — Fresh, Gm.	Dry, Gm.	Capsules — Fresh, Gm.	Dry, Gm.
First Generation[b]										
Control	105.0	21.5	46.0	11.0	31.5	7.0	27.5	3.5	...[d]	...[d]
Amo-1618	116.6	23.2	51.8	11.5	31.0	7.1	33.8	4.6	...[d]	...[d]
MH	112.5	24.2	51.0	14.0	31.5	6.7	30.0	3.5	...[d]	...[d]
Second Generation[c]										
Control	179.7	26.4	60.3	6.2	72.8	13.8	20.1	2.76	26.5	3.71
CGA	173.4	25.8	56.0	5.9	75.3	14.3	20.5	2.72	21.6	2.90
Amo-1618	173.0	24.2	57.0	5.5	73.3	12.8	20.2	2.81	22.6	3.03
AGA	174.6	25.0	53.7	5.4	73.8	13.6	23.3	2.73	24.3	3.34
MH	179.3	26.3	55.8	5.9	75.7	14.3	22.8	2.59	25.0	3.54
MGA	186.1	27.6	56.8	5.8	77.7	14.8	22.1	2.76	25.5	4.33

[a] First generation plants mean weights based per group of six plants; second generation, per group of 10 plants. [b] First generation plants were 53 days old when harvested. [c] Second generation plants were 67 days old when harvested. [d] The capsules were included with the leaf-top of the first generation.

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

Treatment	Leaf-Tops — Alkaloids, mg./Gm.	Controls, %	Stems — Alkaloids, mg./Gm.	Controls, %	Roots — Alkaloids, mg./Gm.	Controls, %	Capsules — Alkaloids, mg./Gm.	Controls, %
First Generation								
Control	5.10	...	2.30	...	1.95	...	...[b]	...[b]
Amo-1618	3.90	76	1.83	80	1.50	77	...[b]	...[b]
MH	4.80	94	2.20	96	2.10	108	...[b]	...[b]
Second Generation								
Control	3.36	...	0.56	...	0.56	...	2.84	...
CGA	3.00	89	0.48	85	0.56	100	2.83	99
Amo-1618	3.44	102	0.60	107	0.48	85	2.60	91
AGA	3.64	108	0.56	100	0.56	100	2.60	91
MH	2.82	84	0.40	71	0.48	85	2.24	78
MGA	3.48	104	0.48	85	0.62	110	3.36	118

[a] Expressed as scopolamine. [b] Capsules of the first generation plants were combined with the leaf-tops.

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

Treatment	Per Plant — Total Alkaloids	Control, %	Leaf-Tops — Total Alkaloids	Control, %	Stems — Total Alkaloids	Control, %	Roots — Total Alkaloids	Control, %	Capsules — Total Alkaloids	Control, %
First Generation										
Control	79.7	...	56.1	...	16.8	...	6.8	...	...[b]	...[b]
Amo-1618	64.4	81	44.8	80	12.7	75	6.9	101	...[b]	...[b]
MH	88.2	111	67.2	120	14.7	87	7.3	107	...[b]	...[b]
Second Generation										
Control	40.5	...	20.8	...	7.7	...	1.5	...	10.5	...
CGA	34.2	84	17.6	84	6.9	89	1.5	100	8.2	78
Amo-1618	35.9	88	19.0	91	7.7	100	1.3	87	7.9	75
AGA	37.5	92	19.7	94	7.6	98	1.5	100	8.7	82
MH	31.4	77	16.6	80	5.7	74	1.2	80	7.9	75
MGA	43.3	107	20.0	96	7.1	91	1.7	113	14.5	138

[a] Calculated from dry weight and alkaloid analyses data; per plant = leaf-tops + stems + roots + capsules. [b] Capsules of first generation plants were included with the leaf-tops.

the per cent of alkaloids determined from the alkaloid analysis and expressing the results in milligrams.

Chlorophyll Determination.—The chlorophyll analysis was conducted on 0.5-Gm. samples of leaf-tops material of only the second generation plants by a method described in a previous publication (23). A minimum of two duplicate determinations was performed on each sample.

Selective Solvent Extraction.—To determine the effects of the treatments on other types of metabolic products, duplicate 2-Gm. samples of powdered leaf-tops material from each of the six series of the second generation were extracted completely in a Soxhlet apparatus in sequence with the following solvents: petroleum ether U.S.P., anhydrous ether C.P., alcohol U.S.P., and distilled water (23). Second duplicate determinations were performed when agreement was not obtained between two samples of each series.

RESULTS

Growth Effects.—In the first generation study, the MH group was about 7% shorter than controls. Growth was affected since the total dry weights of

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

Treatment	Petroleum Ether	Ether	Alcohol	Water
Control	115	6	199	198
CGA	112	7	136	215
Amo-1618	105	14	159	189
AGA	101	14	202	193
MH	100	20	163	183
MGA	115	10	201	179

[a] Based on 2-Gm. samples; expressed as milligrams per gram.

the treated groups were about 10% greater than controls (Table I). The dry weight increases of 27% in the leaf-tops (the aerial parts of the plant exclusive of the stems) of the MH group and 31% in the roots of the Amo-1618 group were considered significant. Otherwise, the general appearance of the plants did not differ appreciably.

The height of the treated groups of the second generation was not significantly affected. The dry weights in the leaf-tops of the Amo-1618, CGA, and AGA series were markedly reduced (Table I). In general, the dry weights of the organs of the MH series were less than controls. GA seed treatment reversed the inhibitory effect on growth since all plant parts of the MGA series weighed more than the MH series. The following conclusions were made from a statistical analysis[3] of the dry weight data. The dry weights of the stems were not significantly[4] affected; the dry weight of the leaves of the Amo-1618 series was reduced significantly[4,5]; the dry weight of the capsules of the MGA series increased significantly[4,5] compared with the control and the MH series. GA seed treatment generally had a stimulatory effect on the weight of plants inhibited by the MH treatment.

Effect on Alkaloid Patterns.—The concentration of the alkaloids in the organs of the Amo-1618 group of the first generation was reduced significantly (Table II). No significant differences from controls were found in the plant parts of the MH group. This phenomenon of reduced concentrations of alkaloids did not carry over into the second generation of the Amo-1618 group, except for the root organ. The concentration of alkaloids in the aerial parts of the CGA series was appreciably lower than controls. Significant reductions in the concentration of alkaloids were noted generally in the organs of the second generation of the MH series. The similar series of the first generation did not display this trend. GA seed treatment of the MH series markedly reversed the inhibitory effect on alkaloid concentration noted in the MH series.

The total alkaloid content of the first generation of the Amo-1618 group was reduced significantly, while that of the MH group was increased markedly (Table III). With the second generation plants, the decrease of the total alkaloid content in the Amo-1618 series was not so great as that noted in the first generation. With the MH series, significant reductions in total alkaloids were noted in all organs. This pattern was the reverse of that found in the first generation. The GA seed treatment of the controls caused a reduction in the total alkaloids of the aerial parts. The residual inhibition noted in the Amo-1618 and MH series was reversed by the gibberellin seed treatment.

Chlorophyll Content.—The chlorophyll analysis of the leaf-tops of the second generation indicated no appreciable differences between the various series of plants.

Selective Solvent Extracts.—The following trends were noted from the selective-solvent extraction of the leaf-tops of the second generation plants. The petroleum ether extract was generally less than the controls for all series; the ether extract was increased twofold in the Amo-1618 and AGA series and about threefold in the MH series; the GA seed treatment reversed the reductions found in the alcohol extract of the Amo-1618 and MH series; the alcohol extract of the CGA series was reduced significantly. No significant trends were found in the water extracts. (See Table IV.)

DISCUSSION

The most characteristic response of sensitive plants treated with proper concentrations of growth inhibitors and retardants is decreased internode elongation (1–6). The plants of the first generation treated with MH were shorter than controls, while those of the Amo-1618 group were about the same height as the untreated group. The anticipated characteristic response did not materialize. The concentrations of the chemicals, which were those suggested in the literature for ornamental plants, were too low to induce a significant retardation in plant height. Nevertheless, this study revealed some noteworthy results.

The Amo-1618 and MH groups of the first generation indicated about a 10% increase in growth (dry weight). The inhibitory effect on plant weight did not appear in the Amo-1618 group until the second generation. This suggests that even though the concentration of the retardant was too dilute to cause inhibition during the first generation, the Amo-1618 was carried over through the seeds into the second generation. It is altogether possible that the embryo and early growth phases of the plants are more susceptible to the growth retardant than are the later stages of plant development. This phenomenon of no appearance of growth retardation until the second generation has been reported by Cathey (1) and Wirwillie and Mitchell (7). Results reported here suggest that the Amo-1618 was translocated into the datura seeds, and the inhibitory effect on growth appeared in the following generation.

In the second generation, the GA seed treatment caused a reversal of the inhibitory effects noted on the alkaloid production of the MH series. This suggests that GA seed treatment in some manner compensates for or counteracts the inhibitory action of MH. Both MH and GA induce their specific growth effects through their action on auxin. Andreae and Andreae (25) found that MH antagonized auxin and suggested that it can bring about increased enzymatic destruction of indole acetic acid (IAA). Galston and McCune (26) indicated that GA acts as an auxin sparing agent

[3] The authors are indebted to Dr. Ahmad A. El-Badawi, Food Technology Department, Oregon State University, Corvallis, for his assistance in conducting the statistical analysis.

[4] At the 95% confidence level.

[5] At the 99% confidence level.

which saves auxin from oxidation by IAA oxidase. Thus, it appears that GA supplied exogenously by the seed treatment may have compensated for or counteracted the MH inhibition through their common influence on auxin.

With the Amo-1618 series of the second generation, GA seed treatment reversed the inhibitory effect noted on the plant weight, the alkaloid content, and the weight of alcoholic extract. This reversal can be explained on the basis of what is presently known concerning the mechanism of action of the quaternary growth retardants, such as Amo-1618 (1). Lockhart (9) and Kende *et al.* (13) demonstrated that these quaternary growth retardants act by blocking the biosynthetic system, providing natural gibberellin to the plant and subsequently termed these compounds "antigibberellins." The reversal of the inhibitory effect noted in the Amo-1618 series by the GA seed treatment could be explained by the fact that the exogenously supplied GA raised the gibberellin level sufficiently to compensate for the block imposed by the growth retardant.

GA seed treatment of the control plants had an inhibitory effect on total alkaloid production. The total alkaloid content of *D. tatula* was reduced about 16% by the treatment. Scott and Sciuchetti (21) reported an 18% reduction in the total alkaloid content of *Atropa belladonna*, and Caldwell and Sciuchetti (22) found a 13% decrease in *D. stramonium* var. *inermis*.

The slight reductions in the petroleum ether extracts of the treated series of the second generation indicates that lipid metabolism and/or accumulation in the leaves was not altered. The ether extract of the Amo-1618 series was increased twofold; that of the MH series was increased threefold. This suggests that the biosynthesis of resinous type components was augmented. The significant reductions in the alcoholic extract of the Amo-1618 and MH series correlates with the decreased total alkaloid content of the leaf-top material.

SUMMARY AND CONCLUSIONS

With the first generation plants, the MH group was about 7% shorter than the controls. The Amo-1618 group was as tall as the controls. In appearance, the treated plants did not differ appreciably from untreated. A favorable effect on growth, about a 10% increase in total dry weights, was noted in the treated groups. Significant reductions in the total alkaloid content per plant were induced by the Amo-1618 treatment. The MH group indicated about an 11% increase in the total alkaloid content per plant.

Significant trends were noted in the second generation plants. Often a reversal of that found in the first generation was observed in the ensuing generation. For the Amo-1618 series, the following trends were considered significant. Leaf-top weight was decreased about 14% compared with controls, contrasted to a 5% increase in the preceding generation; the total alkaloid content per plant of the first generation was decreased about 19%, compared with a 12% reduction in the second generation; about a twofold increase was found in the ether extract; a significant decrease was found in the alcohol extract.

The following pertinent points were observed in the MH series. The total dry weight of the first generation was increased about 12%, while that of the second generation approximated the controls; the total alkaloid content per plant increased about 11% in the first generation, compared with a 23% reduction in the second generation; a threefold increase was found in the ether extract; a significant decrease was noted in the alcoholic extract.

The GA seed treatment generally reversed any inhibitory effects indicated by the chemicals during the second generation. This was shown in the following cases. The total dry weights of the Amo-1618 and MH series (both were less than controls) were greater in each of the series receiving an additional GA seed treatment; the significant reductions in the concentration of alkaloids in the aerial parts of the MH series was reversed in the MGA series; the same pattern was noted concerning the total alkaloid content per plant; significant decreases were found in the alcoholic extracts of the Amo-1618 and MH series, whereas the AGA and MGA series of plants were the same as controls.

The GA seed treatment of controls caused an appreciable decrease in the concentration of alkaloids in the aerial parts and in their total alkaloid content. A 12% reduction was found in leaf-tops dry weight. A significant decrease was found in the alcoholic extract of the leaf-tops. With this series and the other treated series, no marked changes in habit were noted. The chlorophyll analysis confirmed the observations that the leaves of treated plants did not appear appreciably different from untreated.

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