AN ABSTRACT OF THE THESIS OF

Asaad Nashed Masoud for the M. S. in Pharmacognosy
(Name) (Degree) (Major)

Date thesis is presented May 9, 1963

Title THE EFFECT OF AMO-1618, MALEIC HYDRAZIDE AND GIBBERELLIN-SEED TREATMENT ON THE SECOND GENERATION OF *DATURA TATULA* LINN.

Abstract approved

(Major professor)

Previous research conducted by the Pharmacognosy department at Oregon State University on *Datura tatula* indicated that AMO 1618 and maleic hydrazide (MH) affected the growth and alkaloid biogenesis of the plant. AMO 1618 treatment increased the fresh and dry weights of the plants, but decreased the alkaloid content. MH treatment resulted in an appreciable increase in plant weight, an increase in total alkaloid content, but a decrease in height.

In view of these interesting effects on plant growth and alkaloid formation, it was decided to continue the study into the second generation. This investigation was conducted on seeds obtained from the various groups of the aforementioned plants, i.e., those plants treated previously with AMO 1618, those previously treated with MH, and

untreated (control) plants. Since the literature indicated that there is an interaction between some growth retardants and gibberellic acid (GA), part of the seeds from each group was soaked in a 50 ppm solution of GA and another portion soaked in distilled water. This procedure provided six series each consisting of ten plants. The six series of plants were randomized in the greenhouse, growth observations were made and the plants were harvested when they were about 67-days-old.

The growth rate, based on height measurements, was not appreciably affected. However, fresh and dry weight data indicated the following trends: the AMO 1618 second generation plants indicated a marked reduction in fresh weights; the GA-seed treatment induced an increase in the fresh weights of this group; a significant decrease was noted in the dry weight of the leaves of this AMO 1618 group; the GA-seed treatment induced a significant increase in the dry weight of the capsules of this group; the GA-seed treatment induced a decrease in the fresh weight of the control group; the GA-seed treatment did not affect the fresh or dry weights of the MH group.

The alkaloid concentration of the AMO 1618 group, as well as those in this group receiving a GA-seed treatment, was not affected. However, considerable residual inhibition was noted in the concentration of alkaloids in the MH group. This inhibition was markedly reversed by GA-seed treatment. The alkaloid content per plant was decreased in the second generation by both inhibitors. The GA-seed

treatment of the control group reduced the total alkaloid content per plant, whereas, GA-seed treatment caused a reversal of the residual inhibition which was induced by each of the growth retardants.

The chlorophyll concentration was not affected by the various treatments. The following pertinent data was obtained from the selective solvent extraction of the leaves: the petroleum ether, alcohol and water extracts were decreased by the residual effect of both inhibitors; this decrease was reversed by GA seed treatment; two- to three-fold increases were noted in the ether soluble fractions of plants treated with the inhibitors; GA-seed treatment induced a decrease in the alcohol extract of the control group.

THE EFFECT OF AMO-1618, MALEIC HYDRAZIDE AND GIBBERELLIN-SEED TREATMENT ON THE SECOND GENERATION OF *DATURA TATULA* LINN

by

ASAAD NASHED MASOUD

A THESIS

submitted to

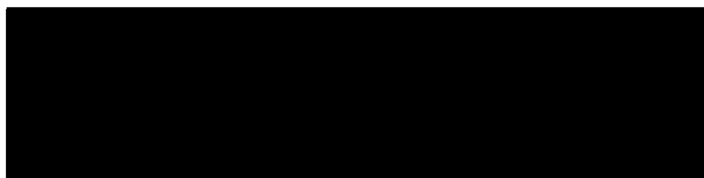
OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

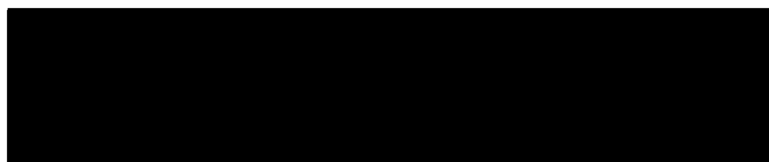
MASTER OF SCIENCE

June 1963

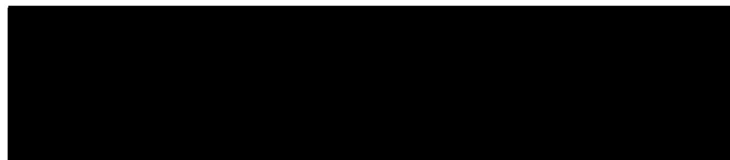
APPROVED:



Professor of Pharmacognosy



Head of Department of Pharmacognosy



Dean of Graduate School

Date thesis is presented May 9, 1963

Typed by Muriel Davis

ACKNOWLEDGMENT

The author is indebted to the following people and wishes to express his sincere appreciation to them:

Dr. Leo A. Sciuchetti for his guidance, valuable suggestions and supervision in the research and preparation of this thesis.

Dr. Philip Catalfomo, and Mr. Norman F. Billups, for their helpful suggestions and criticisms during the preparation of the text of the paper.

Dr. Ahmad A. El-Badawi for his advice in selecting the method of statistical analysis and his help in calculating the data.

Mr. James. H. Bennett and Mr. George H. Constantine for their assistance in harvesting the plants.

The Government of the United Arab Republic for giving me the opportunity and providing me with financial assistance to do this work.

TABLE OF CONTENTS

Chapter		Page
I	INTRODUCTION	1
	Maleic Hydrazide	1
	Mechanism of Action of Maleic Hydrazide	4
	Maleic Hydrazide and Gibberellic Acid Interaction	5
	AMO 1618	6
	AMO 1618 and Gibberellic Acid Interaction	10
	Gibberellic Acid	11
	Chemistry and Action of Gibberellic Acid	11
	Dormancy and Seed Germination in the Solanaceae	12
	Effect of GA on Alkaloid-producing Plants	14
II	PREVIOUS WORK ON FIRST GENERATION	16
	Experimental	16
	Plant Material	16
	Treatment of Plants	16
	Harvesting of Plants	17
	Results	17
III	EXPERIMENTAL	22
	Plant Material	22
	Treatments	23
	Germination	23
	Plant Growth	24
	Care and Measurements	25
	Harvesting of Plants	26
	Alkaloid Analysis	27
	Chlorophyll Determination	29
	Selective Solvent Extraction	32

Chapter		Page
IV	RESULTS AND DISCUSSION	35
	Effect on Height	35
	Effect on Fresh Weights	36
	Effect on Dry Weights	39
	Statistical Analysis of Dry Weights	41
	Effect on Alkaloid Concentration	47
	Chlorophyll Data	53
	Data of Selective Solvent Extraction	53
V	SUMMARY AND CONCLUSIONS	57
	Growth Pattern	57
	Fresh Weights	57
	Dry Weights	58
	Alkaloid Concentration	58
	Alkaloid Content	58
	Chlorophyll	59
	Selective Solvent Extraction	59
	BIBLIOGRAPHY	60

LIST OF FIGURES

Figure		Page
1	Chemical structures of maleic hydrazide, AMO 1618 and gibberellic acid.	2
2	Effect of various concentrations of AMO 1618 on chrysanthemum	7
3	Height measurement of first generation.	19
4	Scopolamine standard curve for alkaloid determination	30
5	Fresh weights resulting from different treatments	37
6	Dry weights resulting from different treatments	40
7	Effects of treatments on alkaloid concentration	50
8	Results of selective solvent extraction	56

LIST OF TABLES

Table		Page
I	Average Weight of Datura Plant Parts	18
II	Alkaloid Concentration of Plant Organs	20
III	Total Alkaloid Content per Plant and per Plant Organ	21
IV	Average Height in cm of Plants During Four-Week Observation Period	35
V	Growth Patterns from Different Treatments as Demonstrated by Height Measurements	36
VI	The Effect of Treatments on Fresh Weight	39
VII	The Effect of Treatments on Dry Weight	39
VIII	Analysis of Variance of Dry Weights of Leaves	44
IX	Analysis of Variance of Dry Weights of Stems	45
X	Analysis of Variance of Dry Weights of Roots	45
XI	Analysis of Variance of Dry Weights of Capsules	46
XII	The Alkaloid Concentration in the Plant Organs	49
XIII	The Alkaloid Concentration of Different Treatments Expressed as Percent of Controls	51
XIV	Total Alkaloid Content of Plant Parts	52
XV	Total Alkaloid Content as Percent of Control	52
XVI	Chlorophyll Content of Leaves of Plants	53
XVII	Weights of Components Extracted by Selective Solvents	55

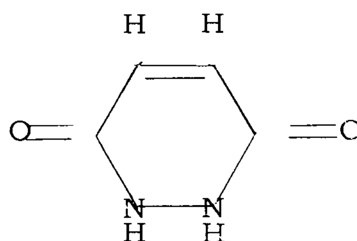
THE EFFECT OF AMO-1618, MALEIC HYDRAZIDE AND GIBBERELLIN-SEED TREATMENT ON THE SECOND GENERATION OF *DATURA TATULA* LINN

I. INTRODUCTION

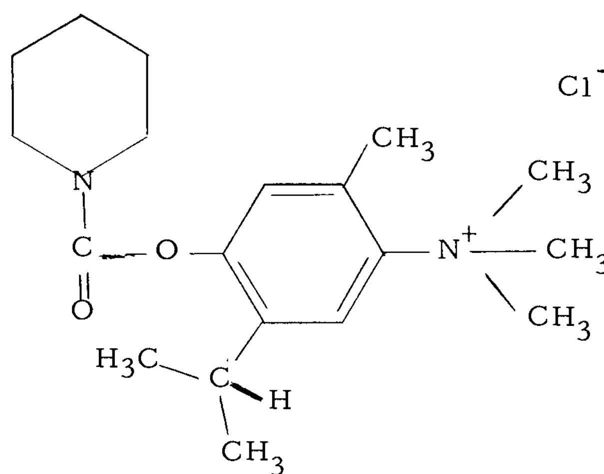
MALEIC HYDRAZIDE

One of the more recent additions to the group of compounds which regulate plant growth is the growth inhibitor maleic hydrazide (MH) (35). Chemically it is 1, 2 dihydro- 3, 6-pyridazinedione (Figure 1)(3,p.166). This compound is not a plant hormone in the conventional sense, but rather seems to be a true growth inhibitor, being unable to stimulate at high dilutions (29; 42). Evidence is accumulating that it acts as an anti-auxin (25). Application of MH to growing plants generally results in dwarfed plants with short internodes and dark-green leaves. MH also induces dormancy, *e.g.* in potato tubers. It breaks apical dominance, causing plants which normally grow by a single main axis to assume a bushy habit. In general, maleic hydrazide inhibits flowering (5).

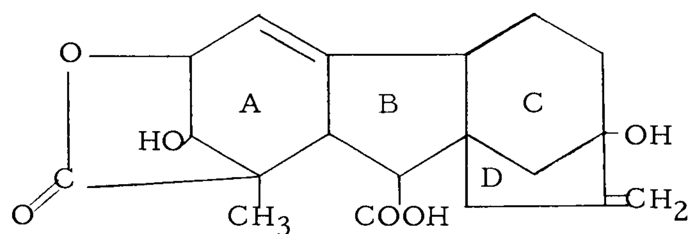
Several studies have been conducted regarding the inhibition of stem growth by maleic hydrazide (13; 35; 44). An investigation with tomatoes (19) demonstrated that MH treatment caused the inhibition of stem elongation. Sachs and Lang (34) studied the effect of MH upon the subapical meristem in *Xanthium sp.* They reported that MH, in appropriate doses containing a total of 0.4 mg. in aqueous



Maleic Hydrazide



AMO 1618



Gibberellic Acid

Figure 1. Chemical structures of maleic hydrazide, AMO 1618 and gibberellic acid.

solution, completely prevents cell division not only in the subapical regions but also in the apical meristem. MH-treated plants, though no longer capable of stem elongation, do not assume a rosette habit of growth because leaf initiation is also prevented. Hence, its action as a regulator of cell division is quite different from that of AMO 1618 or gibberellic acid (GA) (Figure 1) since it lacks the selectivity of the latter two substances.

Baker (4) showed that MH, at concentrations of 0.01 Molar and higher, inhibited oxygen uptake by tobacco tissues to varying degrees, depending on the pH of the solution bathing the tissues. At pH values near 7.0 an inhibition of 10 to 20 percent occurred during the first hour after vacuum infiltration of the tissues by solutions containing MH, whereas at pH 4.0 the inhibition was 30 to 50 percent. Baker also studied the effect of MH on various plant dehydrogenases by a direct method. A general inhibition of dehydrogenases, as postulated by others, was not ascribed to MH since none of the ones tested were inhibited. No inhibition by MH of cytochrome c reductase, cytochrome c oxidase, and polyphenol oxidase, was observed. An inhibition of a diaphorase similar in magnitude to that of respiratory inhibition was demonstrated.

Ermalaeva and Kolzlova (15) investigated the effect of MH on the rate of photosynthesis and on the carbohydrate metabolism of

plants. When *Perilla acymoides* was sprayed once with a solution of maleic hydrazide, vernation of reproductive organs was observed when the day was longer than 16 hours. When the plant was sprayed with a 0.25 percent solution of MH the intensity of photosynthesis was increased five days later. This increase continued for the next 15 days. The assimilatory activity of leaves was accompanied by considerable changes in the morphological structure of leaves. It appeared that MH treatment increased the photosynthetic capacity of leaves.

Mechanism of Action of MH

The fact that it broke apical dominance as well as inhibited growth suggested that MH might lower auxin effectiveness. Experiments indicate that it inhibits growth in standard auxin assays, and that its inhibitory effects can be removed by the presence of additional auxin. Conversely, the inhibitory effects of excess auxin can be reversed by addition of maleic hydrazide (25). The reduction of auxin effectiveness by MH may be explained in part by the finding that it can bring about increased enzymatic destruction of indoleacetic acid (2). A synergistic effect with auxin has also been reported (17).

Maleic Hydrazide and Gibberellic Acid Interaction

There is some controversy in the literature on the interaction of MH and GA. Bukovac and Wittwer (8) reported that GA overcame the inhibitory effects of MH on the epicotyl growth of beans. Kato (23) showed that MH-induced inhibition of shoot growth in cucumber seedlings was partly prevented by GA. Brian and Hemming (5), working with a variety of peas not responding to GA, concluded that GA did not reverse MH-induced inhibition of stem growth but that MH probably interfered with the normal growth response at some stage before GA exerts its effect. They interpreted Bukovac and Wittwer's experiments, as well as their own, to mean that MH reduced or prevented the response of GA-sensitive plants to GA. GA does not reverse MH-induced inhibition of cell division, yet the story may be quite different where cell expansion is involved. For this reason it is still difficult to assess MH-GA interactions in the over-all growth of plants.

Lockhart (26) has shown that there is no interaction between MH and the gibberellins. In contrast to this he has shown that AMO 1618 does competitively inhibit gibberellin and therefore is a true anti-gibberellin.

AMO 1618

A recently introduced plant growth inhibitor (11) known chemically as (4-hydroxy-5-isopropyl-2-methylphenyl)-trimethylammonium chloride-1-piperidine carboxylate, is called AMO 1618 (Figure 1). The compound inhibits stem elongation without causing malformation or toxicity. It keeps most varieties of potted chrysanthemums short and miniature in size. AMO 1618 produces more attractive potted plants, and its use has great potential in the production of small potted plants for mass markets. This product may open up an entirely new field of production and marketing for dwarf types of plants.

The main work conducted by the Rainbow Chemical Company was on chrysanthemums (32). AMO 1618 delayed flowering in these plants up to two or three weeks. The plants must be sprayed during floral initiation, which is during the first two weeks of short days. If applied after the flower is formed, little or no delay results. It suppresses development of the florets (Figure 2).

Sachs and Lang (34) found that AMO 1618 inhibited stem elongation in caulescent plants. Extensive work by the U. S. D. A. on quaternary ammonium carbamates demonstrated that these substances, the most readily available and most active of which was

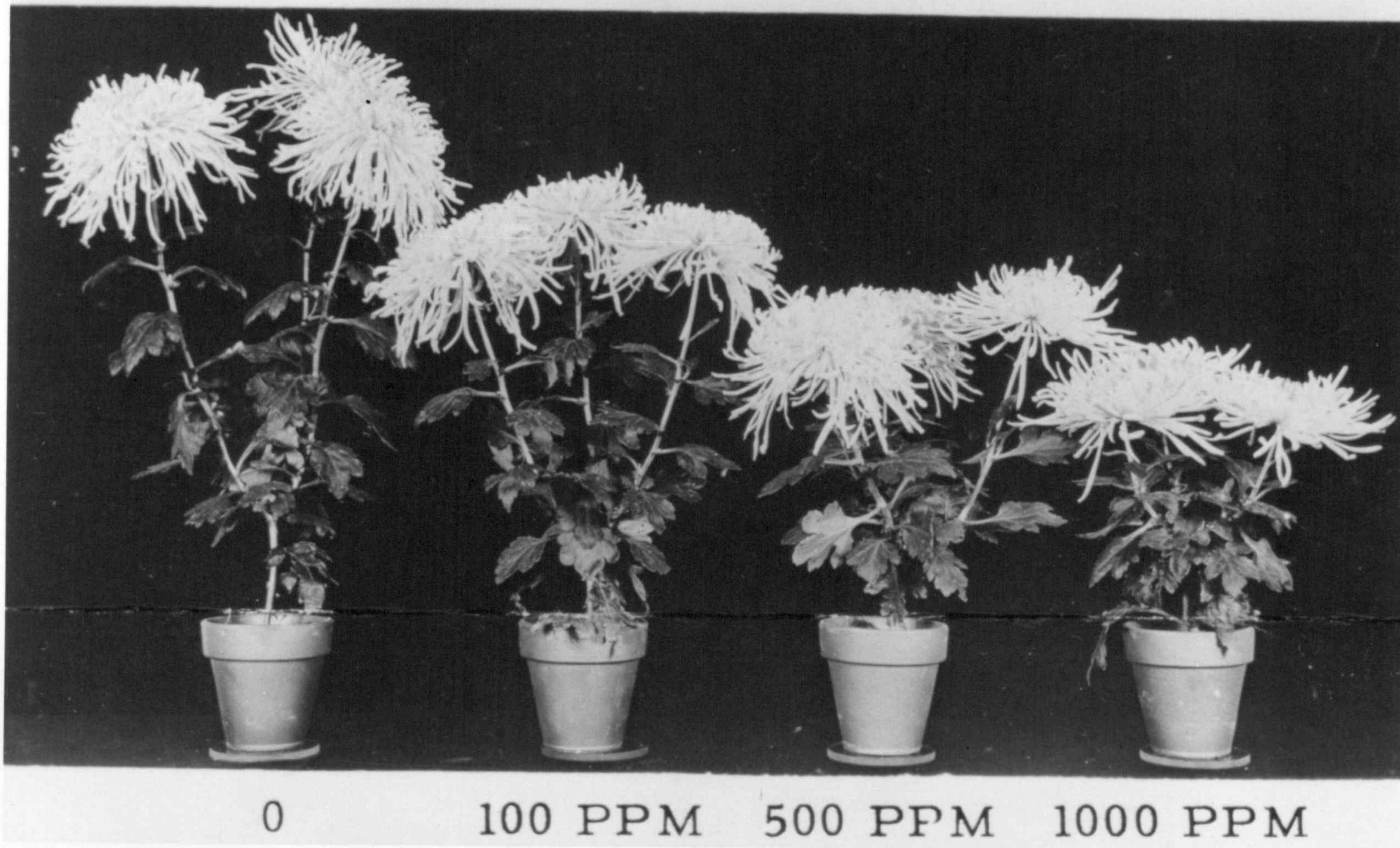


Figure 2. Effect of various concentrations of AMO-1618 on chrysanthemem.

AMO 1618, caused a striking inhibition of stem elongation in many plants. It was of particular interest that GA reversed the inhibition induced by AMO 1618 in chrysanthemum. Subapical (but not apical) cell division was considerably reduced by treatment with AMO 1618 and, soon thereafter, shoot elongation ceased. Since the apical meristem functioned normally or almost so, and leaf initiation was continuing; the treated plants assumed a dwarf or rosette habit of growth. GA prevents and reverses inhibition of subapical cell division induced by AMO 1618 (34).

Marth and Mitchell (27) designed an experiment to determine the length of time that AMO 1618 persists in soil as indicated by the response of plants grown from seeds sown therein over a period of successive years. It was noted repeatedly in the course of the nine-year experiment that plants which had shown but slight growth retardation from AMO 1618 treatment were the most resistant to microorganisms causing foliar discoloration and death of the plant. Plants that received rather strong dosages of AMO 1618 developed leaves that were relatively thick and leathery. This might have presented a physical barrier to the invasion of microbes. The chemical is readily translocated upward in plants and it is possible that at the higher dosage levels applied to the soil there was a systemic effect on the plant. This resulted in a retardation of the

growth of microorganisms that would otherwise have attacked the plants.

Cathey and Stuart (11) compared the activity of AMO 1618 Phosphon (2, 4-dichlorobenzyltributyl phosphonium chloride) and CCC (2-chloroethyl trimethylammonium chloride) on 55 species of plants. The plants were given soil and foliar application of the growth retardants. Treatment with AMO 1618 resulted in retarded growth of only six species; while different responses were reported for the other inhibitors. The side effects of the three chemicals were different even though the primary action (retarding internode elongation) was similar. AMO 1618 induced few side effects, persisted in the soil, and affected more than one crop of plants. A seasonal variation in response to the chemicals was observed. Growth retardation following application of AMO 1618 was slightly greater in the summer.

AMO 1618 causes numerous effects in plants. Germination of seeds was delayed. Root development was initially inhibited, but effects were seldom apparent at flowering time. Shorter internodes were noted on most plants; the main stem of certain treated plants grew in essentially a rosette fashion. Leaves on most treated plants were much darker green than those on untreated plants. Varying degrees of chlorosis followed excessive treatments. Blade

expansion was often slow, but the leaves eventually attained a similar size to those of untreated plants. Control of flower initiation by photoperiod and temperature was unaffected; although flowering time of most plants was not noticeably altered. Flowering of chrysanthemum and cleome was delayed. Flower bud initiation of azaleas was promoted. The reduction in fresh and dry weights observed for treated plants resulted primarily from the reduced stem length.

AMO 1618 and Gibberellic Acid Interaction

Marth and Mitchell (27) concluded that gibberellin and AMO 1618 applied as a lanolin paste were mutually antagonistic on the stem elongation of pinto beans. The photoperiod controlled effects of gibberellin and AMO 1618 on the growth and flowering of *Chrysanthemum morifolium* Ramat were studied by Cathey (12). Mutual antagonism of gibberellin and AMO 1618 on the growth and flowering of chrysanthemums, short photoperiodic plants, was noted. Gibberellin alone induced maximum elongation when applied in the third week of short photoperiods while AMO 1618 alone induced maximum suppression of growth when applied at the start of short photoperiods. They were mutually antagonistic when applied simultaneously four times in the first two weeks of short photoperiods.

GIBBERELLIC ACID

The amazing effects of the gibberellins on plant growth have been well established due to the extensive number of investigations reported during the past eight years (38).

There is a voluminous literature on the effect of GA on various plants. The literature has been adequately reviewed by many authors (7; 38 ; 39; 40; 41; 43). The influence of this growth regulator on medicinal plants has been investigated during the past five years and has been reviewed by Ambrose and Sciuchetti (1).

Chemistry and Action of Gibberellic Acid

Gibberellin is the generic name of several closely related chemical substances which exert profound effects on plant behavior. Presently, seven gibberellins have been characterized and identified which are designated as A_1, A_2, A_3, A_4, A_5, A_7, and A_9. Gibberellin A_3 proved to be identical with a substance, reported as gibberellin x, and is now known as gibberellic acid (GA). GA has been employed by most investigators since it is believed to be the most active of the gibberellins and a relatively pure form is available.

It was suggested that gibberellins or gibberellin-like native growth substances were factors limiting the subapical meristematic

activity in rosette plants. Thus, the transition from the rosette habit of growth to stem elongation is the result of cell division in the subapical region of the shoot and this process is controlled by gibberellin.

Sachs and Lang (34) concluded that GA-induced stem elongation in rosette plants by stimulating mitotic activity immediately below the apical meristem. The cells produced in this zone after elongation, constitute the tissues of the mature, elongate stem. In caulescent plants a subapical zone of mitotic activity was observed as much as two cm in length, similar in activity to that in rosette plants receiving prolonged treatment with GA. It would appear from the reports in the literature on the action of GA in the cell that both cell extension and cell division play an important role in GA-induced growth. The recent work of Sachs and Lang indicate that cell division is implicated to a greater extent than heretofore believed.

Dormancy and Seed Germination in the Solanaceae

The dormancy of potato tubers is broken by treatment with GA. This type of dormancy is not related to photoperiod or any requirement for vernalization, but disappears gradually following the harvest of the tubers. Earlier and more uniform sprouting which results in a more rapid maturity of the crop has resulted from treatment of both dormant and non-dormant tubers (6).

Treatment of seeds with GA has, in many cases, overcome seed dormancy and has promoted an earlier and more uniform emergence of the seedlings. Work at Oregon State University (38) has indicated that seeds from stramonium plants treated with GA germinated more rapidly and that a more uniform stand was obtained. Similar trends were noted in gibberellin-treated seeds from *Atropa belladonna*, *Hyoscyamus niger* and *H. albus* (37).

Caldwell and Sciuchetti (10) conducted an experiment on the seeds of *Datura stramonium*. A three-fold increase in germination was noted in the treated seeds. Throughout the observation period the habit of these plants closely resembled the controls except that they were slightly shorter than plants grown from untreated seeds. It was noted from dry weight data that during the early stages of growth the treatment induced a deleterious effect. However, no significant change was observed in weights at the final harvest. The concentration of alkaloids in the plant organs was not altered markedly. Due to the decreased growth the total alkaloidal content of the plant at the first two harvests was considerably reduced in the treated group. However, no significant difference was observed at the final harvest. The seed treatment was considered beneficial since a greater and more uniform emergence of seedlings was obtained while the total alkaloid content per plant was reduced only

slightly at the final harvest.

Effect of GA on Alkaloid-producing Plants

Among medicinal plants the most intensive investigations have been done with those producing alkaloids. Thus far, the members of the Solanaceae have received the most attention. Curry and Wassink (14) reported that GA substituted for the long-day requirements necessary for the flowering of annual Hyoscyamus niger. Lang (24) made the important discovery that GA substituted for the cold treatment needed by the biennial plant to flower. Lang's results have since been generally confirmed with other cold-requiring long-day plants.

From a review of the literature (1; 7; 16; 28; 38 ; 39) characteristic gibberellin-effects have been noted in solanaceous plants. GA has been shown to induce taller and spindlier plants, greater internodal elongation, slight chlorosis of the leaves and stems and decreased concentration of alkaloids in the aerial parts. The above effects have been reported in Datura stramonium (7; 16 ;38 ;39), Datura innoxia (22), Atropa belladonna (7; 39), Hyoscyamus niger (39), Nicotiana sp. (9; 28), and Datura meteloides (1).

Ragan and Sciuchetti studied the effect of GA on the second generation of Datura stramonium (31). It was concluded that the

"gibberellin effect" did not manifest itself in the second generation plants. Likewise, gibberellin-effects were not noted in the second generation of *Atropa belladonna* (36).

In view of the fact that AMO 1618 and MH had growth retarding effects on many plants and since the effects of these chemicals had not been previously investigated, the Pharmacognosy department (20; 21) at Oregon State University decided to determine the influence of these growth regulators on the growth and subsequent alkaloid formation in *Datura tatula*. The first phase, which was done by other workers is presented in Part II of this thesis (phase I of the overall experiment). The results of the first part of the experiment indicated that further work on the second generation plants would be desirable. This phase (part II of the overall experiment), described in Part III, was performed by the author.

II - PREVIOUS WORK ON THE FIRST GENERATION

EXPERIMENTAL

Plant Material

The first phase of this experiment was started on April 14, 1960, by Hisatomi and Sciuchetti (20; 21). The *Datura tatula* plants used were selected from seedlings grown in flats at the greenhouse at Oregon State University. The plants were then transplanted into containers (one-gallon cans with drainage holes at the base) that had been filled with a sandy loam mixture. Approximately five grams of complete organic fertilizer[1] was previously mixed into the soil of each container. The cans were randomized and placed on a bench in the greenhouse.

Treatment of Plants

The purpose of the first phase of this investigation was to determine the effects of two growth retardants, MH and AMO 1618, on the growth pattern and alkaloid accumulation in *Datura tatula*. The chemicals were administered in the form of a spray with a nebulizer

---

[1] Organic Morcrop®, Chas. Lilly Co., Seattle, Wash. Analysis: 5% of total nitrogen, 3% available phosphate, 2% available potash.

to the youngest leaves of the plants. Three sprayings of approximately 0.1 ml each of a 100 ppm solution were given to each plant. The MH series was treated once weekly; the AMO series, twice weekly; the controls were not treated. Treatments commenced on April 26, 1960, and were continued until plants were harvested. Height measurements were taken twice weekly.

Harvesting of the Plants

The 53-day-old plants were harvested on June 6. Each plant was thoroughly washed and then blotted to remove excess water, and divided into three portions: leaves-tops, stems and roots. Each of the morphological parts was immediately weighed to determine fresh weight. The respective plant parts were then dried in a hot-air circulating dryer at approximately 48° C. for 48 hours. Then the dry weights were determined. The plant parts were pooled according to treatment, powdered in a Wiley mill and stored in glass containers until needed for subsequent analysis. The alkaloid content was determined by the Brummett-Sciuchetti method (7).

RESULTS

The leaves of both the AMO 1618 and MH treated plants appeared slightly chlorotic when compared with control plants.

Otherwise, the treated plants appeared as healthy as the controls. The average heights of plants treated with AMO 1618 paralleled that of the controls, whereas, those treated with MH were shorter (Figure 3).

Table I summarizes the fresh and dry weight data. The total fresh weight of the treated plants was considerably greater than controls. A similar trend was noted in the dry weights. However, a marked increase was noted in the dry weight of the leaves and tops of plants treated with MH. Root dry weight was increased in both treated groups.

TABLE I

AVERAGE WEIGHT* OF DATURA PLANT PARTS

	Leaves and Tops		Stems		Roots		Total Wt./Plant	
Treatment	Fresh Gm	Dry Gm	Fresh Gm	Dry Gm	Fresh Gm	Dry Gm	Fresh Gm	Dry Gm
Control	46.0	11.0	31.5	7.0	27.5	3.5	105.0	21.5
Maleic Hydrazide	51.0	14.0	31.5	6.7	30.0	3.5	112.5	24.2
AMO 1618	51.8	11.5	31.0	7.1	33.8	4.6	116.6	23.2

* Based on the average of six plants per group.

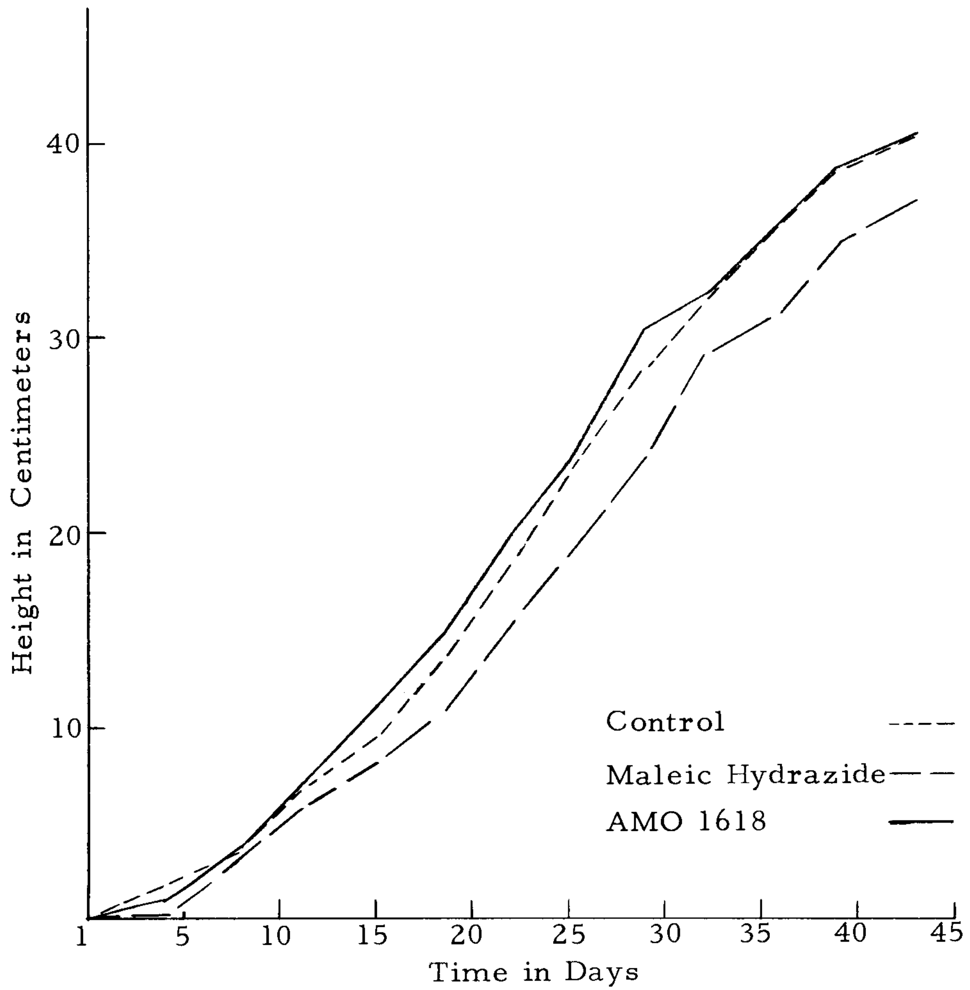


Figure 3. Height measurement of first generation.

The concentration of alkaloids in the leaves-tops of the AMO 1618 group was significantly reduced (Table II). The concentration in the stems was less in the treated groups than in controls, whereas, that of the roots showed variable trends. The total alkaloid content, based on the alkaloid analyses and dry weight data, was generally increased in the MH group but decreased in the AMO 1618 group (Table III).

TABLE II

ALKALOID CONCENTRATION OF PLANT ORGANS

Treatment	Leaves and Tops mg/Gm	Stems mg /Gm	Roots mg /Gm
Control	5. 1	2. 3	1. 95
Maleic Hydrazide	4. 8	2. 2	2. 1
AMO 1618	3. 9	1. 8	1. 5

TABLE III

TOTAL ALKALOID CONTENT* PER PLANT AND PER PLANT ORGAN

Treatment	Whole Plant mg	Whole Plant Percent of Control	Leaves and Tops mg	Leaves and Tops Percent of Control	Stems mg	Stems Percent of Control	Roots mg	Roots Percent of Control
Control	79.7	-	56.1	-	16.8	-	6.8	-
Maleic Hydrazide	88.2	110	67.2	117	14.7	87	7.3	107
AMO 1618	64.4	79	44.8	78	12.7	75	6.9	101

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

III - EXPERIMENTAL

This research (the second phase which was conducted by the author) was a continuation of the work previously mentioned. The purpose of this phase of the project was to determine whether the effects of the growth retardants which were noted in the first generation would be carried over into the second generation of plants. Further, since the literature indicates that there is a possibility of interaction between GA and some growth retardants, it was desired to ascertain whether GA would overcome growth inhibition if it occurred in plants grown from seeds obtained from plants which had been previously treated with growth retardants.

Plant Material

The seeds which were used in this study were obtained from Datura plants which had received in 1960 as previously described: (a) treatment with AMO 1618 (A series), (b) treatment with MH (M series), and (c) untreated plants (controls or C series). The seeds from each of the plants described in the previous study were pooled according to the above described plan and were thoroughly mixed together according to series so as to assure representation of all different genotypical recombinations within each treatment.

Treatments

Two hundred seeds from each series were divided into two groups of 100 seeds. One group was soaked in a vial containing 30 ml of distilled water. The other group was soaked in a vial containing 30 ml of a 50 ppm solution of GA[2]. The seeds were allowed to soak in the solutions for 48 hours. It was noted that most of the seeds had settled to the bottom of the vials which indicated almost complete imbibition of the solutions. This procedure provided six series of plants for this phase of the experiment, viz., series A, plants grown from seeds of plants treated previously with AMO 1618; series AGA, plants grown from series A seeds treated further with GA; series M, plants grown from seeds of plants treated previously with MH; series MGA, plants grown from series M seeds treated further with GA; series C, plants grown from seeds of untreated plants; series CGA, series C seeds treated further with GA.

Germination

On July 7, 1962 the seeds were germinated in flats containing a mixture of one part of sand and two parts of loam. About 50 Gm

[2] GA (10 percent powder), 50 mg of this powder was diluted in 100 ml distilled water. The GA was furnished through the courtesy of Dr. Edwin F. Alder, Plant Physiologist, Agricultural Research Center, Eli Lilly and Co., Greenfield, Indiana.

of Organic Morcrop was previously mixed thoroughly into the soil of each flat. Each of the flats was divided into six rows. Fifty seeds were planted in each row and the seeds and the series according to treatment were arranged at random. The flats were covered with glass plates to provide optimal germinating conditions. The glass was removed after germination and after satisfactory seedling growth had been attained. Germination took place after about a week. Some of the seedlings showed damping-off symptoms. All the flats were treated with a fungicide called Ferbam[3]. The soil was kept moist by daily waterings.

Plant Growth

On July 23, 1962, 20 representative seedlings were selected from each series and were transplanted into paper bands filled with the previously mentioned soil mixture. The paper bands were arranged in flats, 20 per flat.

On August 7, ten uniform plants were selected from the 20 of each of the six series. Each plant was transferred to a one-gallon tin can and filled with the previously described soil mixture to

---

3 Ferbam Wettable Powder Fungicide®: made by the General Chemical Division of the Allied Chemical and Dye Corporation, 40 Rector St. New York 6, New York.
Analysis: 76% ferric dimethyldithiocarbamate
24% inert ingredients.

which had been thoroughly incorporated five Gm of organic fertilizer.

The plants were randomized by arranging the 60 cans in 12 rows of five each. Sixty stakes properly labeled to indicate 10 plants for each of the six series were placed in a carton box and were thoroughly mixed. Then the stakes were picked at random, one by one and placed in the cans. The plants were then transplanted into the cans according to the particular treatment indicated by the stake in the can. In this manner the plants from different treatments were homogenously randomized.

Care and Measurements

The plants were watered daily. In order to minimize infestation with the red spider (Tetranychus telarius) and aphids, all plants were dusted every other day during the infestation with Malathion Powder (4% Malathion Dust) which was applied to the lower surface of the leaves where the spiders and aphids live and lay their eggs. This insecticide appeared to be satisfactory to overcome the aphid infestation but it was not very effective against the red spider. Miller's Tetradane[4] spray was also applied. It was used as a

[4] Miller's Tetradane ornamental and rose spray®

Active ingredients:	
Malathion (0.0 - dimethyl dithiophosphate of diethyl mercapto succinate)	10.00%
0,0 diethlyl-0- (2 isopropyl - 4 methyl - 6 pyrimidinyl phosphothionate	3.40%
1,1 - bis (chlorophenyl) 2, 2, 2 - trichloroethenol	3.00%
DDT (Dichloro Diphenyl Trichloroethane)	7.78%
Lindane, Gamma isomer of B. H. C.	4.00%
Diethyl Diphenyl Dichloroethane	7.78%
Related Reaction Products	0.41%
Aromatic Petroleum Derivative Solvents	13.60%
Inert ingredients	50.03%

diluted oily emulsion in water, sprayed on the lower surface of the leaves. These precautionary measures satisfactorily controlled the insect infestations.

Height measurements were commenced on August 13, 1962, when the plants were about 37-days-old. The height, in centimeters, was taken from the base of the stem at soil level to the highest leaf. Measurements were taken twice weekly, on Mondays and Thursdays at about the same time in the morning, throughout the observation period.

Harvesting of Plants

All of the plants were harvested, on September 13, 1962. At that time the plants were 67-days-old. The plants were removed from the soil by using water at relatively high velocity to free the whole root system. The whole plant was washed carefully to remove the dirt, the insecticides and other residues that remained on the plant parts. They were then separated into four plant parts; leaves and tops, capsules, stems, and roots. Each plant part was blotted carefully and weighed immediately to determine the fresh weight; then it was labeled and wrapped in a light piece of muslin and arranged in a large tray designed to fit the dryer. These plant parts were dried in a circulating hot-air dryer at approximately 48° C.

for 48 hours. The dry weight of each plant part was recorded separately. The plant parts from each treatment were pulverized in a Wiley mill to a #60 powder. The pulverization was done immediately after taking the dry weights. The plant parts from each series were pooled and stored in airtight, colored glass containers. In order to assure complete mixing of each pooled sample, each container was tumbled in a rotating tumbler for 15 minutes. Previous to each analysis the containers were stored in a desiccator for at least 48 hours.

Alkaloid Analysis

The alkaloid analysis was conducted by a method described by Brummett and Sciuchetti (7). The procedure was as follows:

1. A small cotton plug was placed in the bottom of the extractor. The 25-mg sample of pooled drug was then placed in the extractor and shaken into a compact column. Another plug was placed gently on the top of the sample.
2. Citric acid buffer (0.4 ml) was placed on the plant material and allowed to macerate for two hours.
3. The extracted aqueous solution was withdrawn directly into a 25-ml separatory funnel containing 15 ml of chloroform. First, a 3-ml portion of citric acid buffer and

then two 1-ml portions of buffer were used to complete the extraction and rinse the extractor.

4. The combined collection of the aqueous phase was made basic to litmus paper by using 28% ammonium hydroxide. The contents of the separatory funnel[5] was then shaken by hand for one minute and allowed to stand overnight.

5. The 15-ml portion of chloroform was then removed and collected in air tight glass containers. The remaining aqueous phase was shaken for one minute, using an additional 10 ml of chloroform. This was then added to the first 15-ml portion.

6. A 10-ml quantity of the extraction solution was placed in an evaporating dish and evaporated on a hot water bath just to dryness.

7. Then 0. 2 ml of fuming nitric acid was added and this was evaporated to dryness.

8. The residue was dissolved in a few ml of acetone, and transferred to a 10-ml volumetric flask. A quantitative

---

[5] The separatory funnels were fitted with Teflon® stop-cocks to avoid the use of stop-cock grease which often interferes with obtaining accurate reproducible results from the subsequent colorimetric determination.

transfer was made by repeated rinsings of small portions of acetone, and a sufficient quantity of acetone was added to the flask to fill it to the 10-ml mark.

9. A volume of 0.1 ml of recently prepared 3% potassium hydroxide in methyl alcohol was added and mixed with the acetone solution.

10. The mixture was thoroughly shaken and at exactly five minutes later the resultant Vitali color produced was measured in a Bausch and Lomb Spectronic 20 Colorimeter using a 550 millimicron interference filter.

11. The results were compared with a standard curve of scopolamine obtained from known concentrations of the alkaloid (Figure 4).

Chlorophyll Determination

The chlorophyll analysis performed entailed the use of a modification (1) of the method used by Gjerstad (18) in his work with *Mentha piperita* Linné. The method was as follows:

1. A 0.5-Gm sample of the leaves from each treatment was accurately weighed and placed in a thimble in the extraction chamber of a Soxhlet extractor.

2. One hundred ml of solvent (20% water in acetone) was

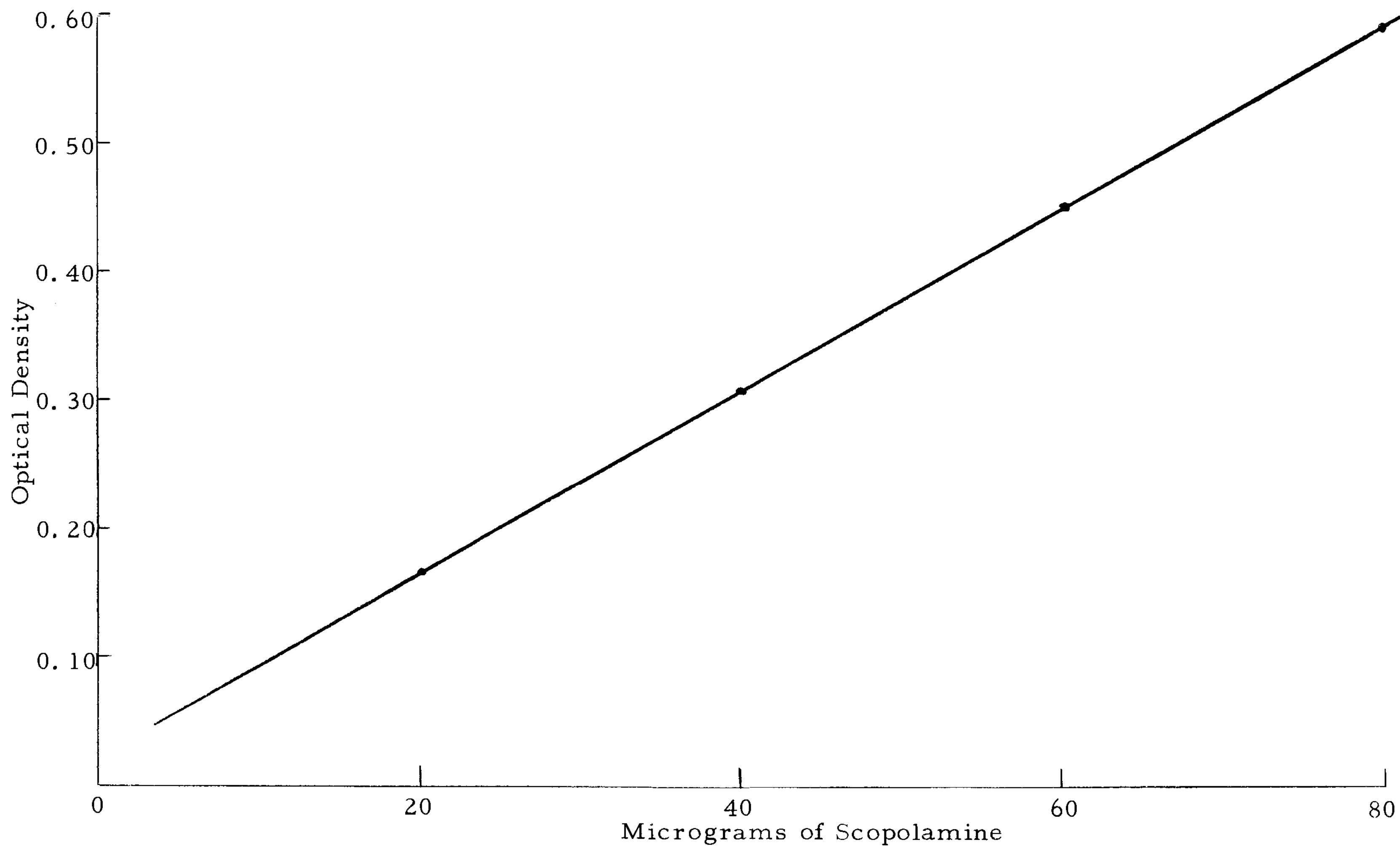


Figure 4. Scopolamine standard curve for alkaloid determination.

used in the extraction process which proceeded for 1 1/2 hours, (a predetermined time necessary for complete extraction) at a rate of approximately one cycle every ten minutes.

3. When the extraction was complete, the chlorophyll-containing solvent was transferred to a 250-ml volumetric flask, and then brought to volume with subsequent washings of the solvent.

4. The optical density of the solution was then read on the Bausch and Lomb Spectronic 20 Colorimeter. Readings were taken at 663 millimicrons and 645 millimicrons, respectively, for each sample. All determinations were made in duplicate.

5. The values thus obtained were inserted in the following simultaneous equations (18) in which x is the concentration of chlorophyll a, and y is the concentration of chlorophyll b. The equations were then solved for x and y.

$$\text{Log} \left[\frac{Io}{I}\right]_{663} = 82.04\ X + 9.27\ Y$$

$$\text{Log} \left[\frac{Io}{I}\right]_{645} = 16.75\ X + 45.6\ Y$$

Selective Solvent Extraction

In order to ascertain the effect of various treatments on other types of components, in addition to alkaloids, it was decided to run the selective solvent extraction.

The principle of the method entails the treatment of the powdered material with a series of different solvents in succession (33, p. 35-40). The decrease in weight in the powdered material represents the amount of solute which is dissolved in each solvent. As a rule each solvent was allowed to percolate until no further substance was extracted. The solvents used successively were as follows:

1. Petroleum ether which may dissolve fats and fatty oils, waxes, essential oils, coloring matters and phytosterols; and less often, alkaloids (present in the free state), glucosides and resinous substances.
2. Absolute ether in turn may dissolve a few glucosides and alkaloidal bases, resinous components and coloring matmer and ether-soluble acids like gallic acid and indifferent bodies.
3. Absolute alcohol will then extract salts (alkaloidal and other salts), saponins or other glucosides of similar behavior, sugars and tannins.

4. <u>Distilled water</u> finally will dissolve some bitter substances and glucosides which may not have been dissolved by the previous solvents. Other substances such as sugars, salts, gums, mucins, proteins, xylans, inulin, and pectins may also be extracted.

The thimbles of the Soxhlet extractor were numbered with pencil and accurately weighed. Two grams of powdered leaf material were accurately weighed and placed in the extraction thimbles. At least one duplicate determination was made for each series. The thimbles were then placed in the Soxhlet apparatus and 100-ml quantities of the solvents were added directly to the Soxhlet flasks. The temperature of each of the Labline hot plates was adjusted so that a regular rate of evaporation could be maintained for each solvent (about one cycle every ten minutes). The extraction was continued until the marc was completely extracted. The times required were about eight hours for the petroleum ether and ether, and about six hours for the alcohol and the water.

After completion of each extraction the thimbles were removed from the apparatus and air dried. The thimbles were then placed in the oven at a temperature of 100° C. and dried to constant weight. No change in weight occurred after 20 hours in the oven. Accordingly the thimbles were dried for 24 hours. When the thimbles were removed from the oven they were placed in a dessicator

until cool and then weighed accurately and quickly to prevent change in weight by absorption of moisture from the atmosphere. In order to determine the weight of material extracted by each successive solvent, the new weight obtained was substracted from the previous recording.

IV. RESULTS AND DISCUSSION

Effect on Height

The plants were measured twice weekly for four weeks. The first measurement was taken when the plants were of a measurable size, which was about five weeks from the average germination date. There were no marked differences obtained in height (Table IV) or growth rate (Table V).

TABLE IV

AVERAGE HEIGHT* IN cm OF PLANTS DURING FOUR-WEEK OBSERVATION PERIOD

Measurement Date	Treatment					
	C	CGA	A	AGA	M	MGA
August 13	13.5	10.8	12.8	13.1	14.1	14.7
" 16	15.6	13.1	14.5	14.9	16.0	16.8
" 20	22.4	18.7	20.6	21.0	21.6	23.6
" 23	29.1	24.7	26.9	27.7	27.9	31.2
" 27	45.3	42.7	44.0	45.6	46.0	49.8
" 30	58.9	55.1	58.0	59.4	59.9	63.9
Sept. 3	76.3	73.6	76.1	77.0	77.0	79.5
" 10	89.1	90.6	93.7	91.7	93.2	92.2
" 12	90.5	90.4	95.0	92.6	93.8	93.2

* Each figure is an average of ten plants.

TABLE V

GROWTH PATTERNS* FROM DIFFERENT TREATMENTS AS DEMONSTRATED BY HEIGHT MEASUREMENTS

Measurement Number & Date	Treatment					
	C	CGA	A	AGA	M	MGA
1, August 13	0.0	0.0	0.0	0.0	0.0	0.0
2, " 16	2.1	2.3	1.7	1.8	1.9	2.1
3, " 20	8.9	7.9	9.8	7.9	7.5	9.9
4, " 23	15.6	13.9	14.1	14.6	13.8	16.5
5, " 27	13.8	31.9	31.2	32.5	31.9	35.1
6, " 30	45.4	44.3	45.2	46.3	45.8	49.2
7, Sept. 3	62.8	62.8	63.3	63.9	62.9	64.8
8, " 10	75.6	79.8	80.9	79.6	79.1	77.7
9, " 12	77.0	79.6	82.2	79.5	79.7	78.5

* The amount of growth in cm after the first measurement, considering the first measurement the zero point.

Effect on Fresh Weights (refer to Table VI and Figure 5)

1. Effect on Leaves. The fresh weights of the leaves of the AMO 1618 treated plants were slightly decreased as compared with controls. MH treated plants also showed slight decreases. The CGA group showed a decrease when compared with the controls; also the AGA group showed less growth than the AMO 1618 group. However, in the case of MGA there was no decrease when compared with the M group.

2. Effect on Stems. The stem fresh weight of all treated groups was higher than the controls (Table VI). The largest increases were noted in the CGA, M and MGA groups. GA seed

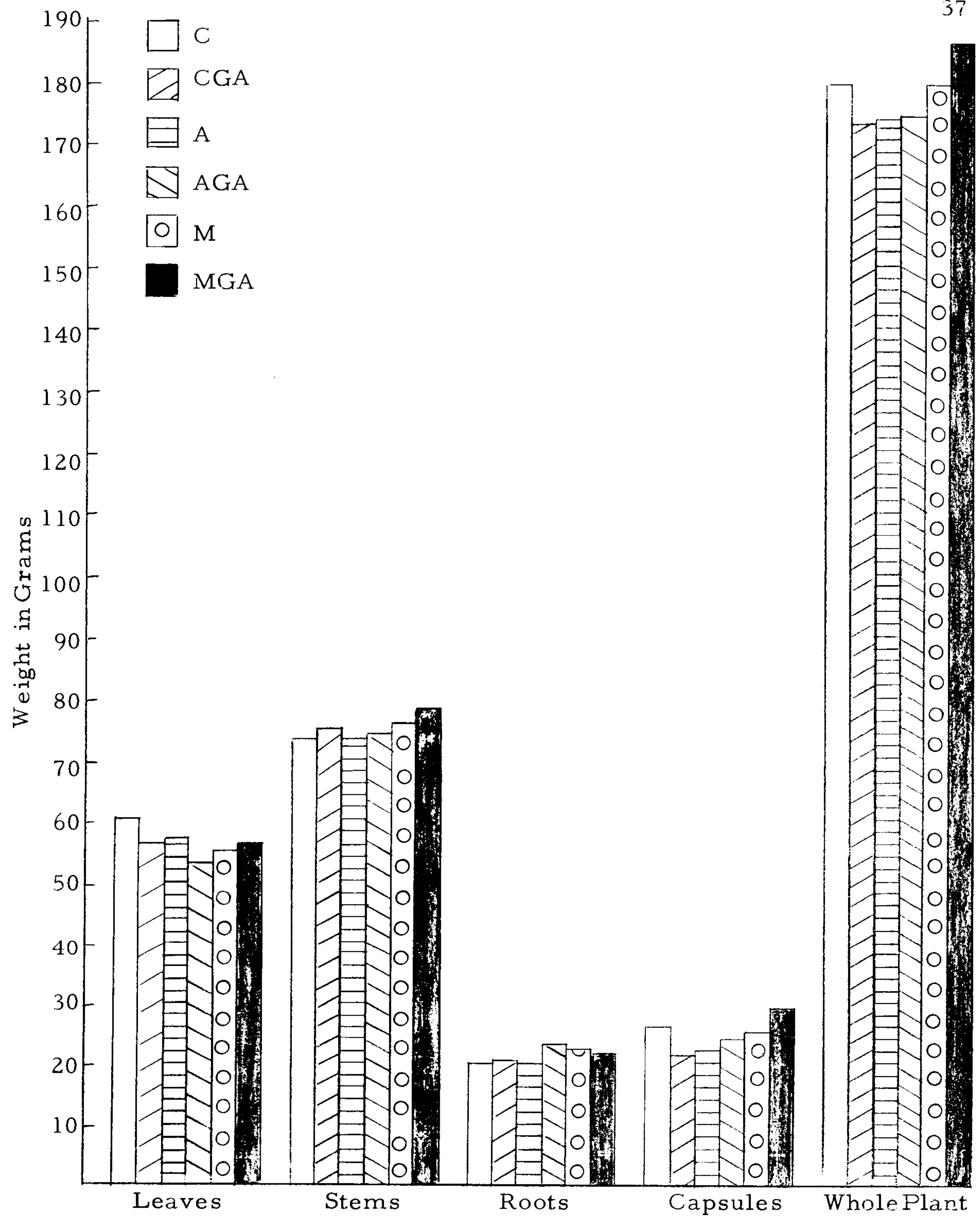


Figure 5. Fresh weights resulting from different treatments.

treatment appeared to increase stem growth in the control and M groups.

3. Effect on Roots. The AGA, M, and MGA groups indicated increased root weights. No significant difference from controls was noted in the remaining groups. Only in the A group was stimulation of growth noted from GA seed treatment.

4. Effect on Capsules. Reductions in the weight of capsules were observed in all the groups except the MGA group. In this case, a considerable increase was noted. GA-seed treatment induced a marked decrease in the controls, but, considerable increases were noted in the AMO 1618 and MH treated plants (Table VI).

5. Effect on the Whole Plant. Total plant weight was considerably greater in the MGA group than in the controls. Decreased plant weight was noted in the CGA, A, and AGA groups. GA-seed treatment appeared to have a stimulating effect in the M group.

In general, the effect of the AMO 1618 and the MH varied in the different plant parts. However, AMO 1618 caused inhibition in total plant growth, while the MH treatment did not cause such inhibition.

The GA-seed treatment elicited an inhibitory effect only in the case of controls, but it caused an increase in weight in the other groups.

TABLE VI

THE EFFECT OF TREATMENTS ON FRESH WEIGHT*

Treatment	Leaves	Stems	Roots	Caps	The Whole Plant
C	60.30	72.80	20.11	26.51	179.72
CGA	56.00	75.30	20.52	21.57	173.39
A	57.00	73.30	20.15	22.59	173.04
AGA	53.70	73.80	23.32	24.26	174.58
M	55.80	75.70	22.80	25.01	179.31
MGA	56.80	77.70	22.06	29.51	186.07

* Each figure represents the average weight in grams of ten plants within the same group.

Effect on Dry Weights

The averages per group of ten plants of the dry weights are shown in Table VII and Figure 6.

TABLE VII

THE EFFECT OF TREATMENTS ON DRY WEIGHT*

Treatment	Leaves	Stems	Roots	Caps	The Whole Plant
C	6.19	13.77	2.76	3.71	26.43
CGA	5.86	14.32	2.72	2.90	25.80
A	5.51	12.84	2.81	3.03	24.19
AGA	5.40	13.55	2.73	3.34	25.02
M	5.90	14.25	2.59	3.54	26.28
MGA	5.77	14.77	2.76	4.33	27.63

* Each figure represents the average weight in grams of ten plants within the same group.

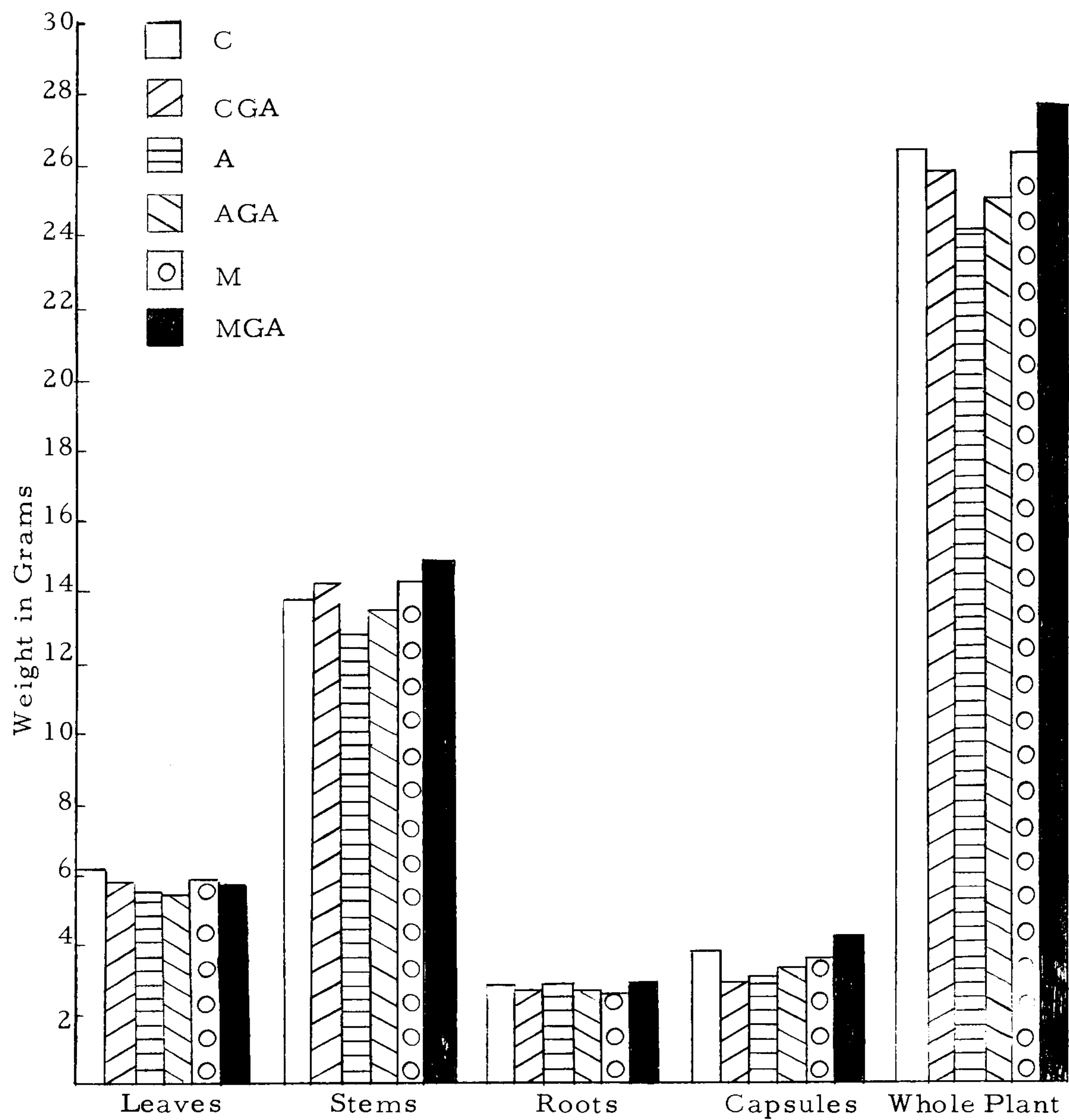


Figure 6. Dry weights resulting from different treatments.

Statistical Analysis of Dry Weights

The dry weight data was analyzed statistically[6] to show the variance within each treatment and between different treatments, and to determine the significance of effects due to any particular treatment (Tables VIII-XI).

1. Leaves (Table VIII). Differences in replications were not significant, but differences between treatments were significant at the five percent significance level. Differences due to individual treatments were calculated as follows:

CGA vs. C: There was no significant difference. This indicates that GA seed treatment did not have an effect on the control plants.

A vs. C: A significant decrease was observed in the one percent significance level. This indicates that AMO 1618 had a significant residual effect on the second generation.

M vs. C: No significant difference was observed. This indicates that there was no residual effect of MH in the second generation.

AGA vs. CGA: No significant difference between these two treatments was observed. This indicates that the residual inhibition of growth by the AMO 1618 was reversed by the GA.

---

[6] The statistical analysis was performed by Dr. A. A. El-Badawi, see acknowledgment.

MGA vs. CGA: No significant difference was observed between these two groups. From this it may be stated that there was no particular significance resulting from combining the MH with GA. Each alone did not show significant difference vs. controls.

2. Stems. The differences in replications were not significant. In addition, the differences between treatments were not significant. This indicates that the six groups did not show a significant difference due to treatments (Table IX).

3. Roots. There were no significant differences within replications or between treatments (Table X).

4. Capsules. There was no significant difference within replications. However, there were significant differences between various treatments at the five percent confidence level (Table XI).

Differences due to individual treatments were as follows:

CGA vs. C: No significant difference; GA did not have an effect on the treated plants when compared with the controls.

A vs. C: There was no significant difference between these two groups. AMO 1618 had residual effect when compared to controls.

M vs. C: There was no significant difference between the two. This indicates that there is no residual inhibitory effect of MH on the second generation.

<u>AGA vs. CGA</u>: There was no significant difference between these two groups. It can be stated that the combination did not bring about a difference in effect as compared to the individual treatment. Hence, neither the individual treatments nor the combined treatments produced an effect.

<u>MGA vs. CGA</u>: There was a significant increase in the MGA combination at the one percent significance level.

Even though there was no significant effect for either MH or GA alone, the combination caused significant stimulation.

<u>Conclusion of Dry Weight Results</u>: The dry weights of the roots and the stems were not affected at all by the treatments. In the case of the leaves and capsules, differences resulted from treatments.

The dry weights of the leaves showed significant decreases due to the residual inhibition of AMO 1618. There were no other significant differences in the leaf data.

The dry weights of the capsules showed a significant increase in the case of the second generation of plants treated with MH whose seeds were treated with GA. There was no other significant effect in the capsules.

The above data indicate that GA seed treatment had a stimulating effect on the plants previously inhibited by MH. However, it did not stimulate the control plants.

TABLE VIII

ANALYSIS OF VARIANCE OF DRY WEIGHTS OF LEAVES

Source of Variation	Degrees of Freedom	Mean Square	F	Remarks* 0.05	0.01
Replication	8	0.1676	0.5895	NS	NS #
Treatment	5	0.7478	2.6303	S	NS
CGA vs. C	1	0.5477	1.9265	NS	NS
A vs. C	1	2.4347	8.5638	S	S
M vs. C	1	0.4050	1.4245	NS	NS
AGA vs. CGA	1	0.7812	2.7478	NS	NS
MGA vs. CGA	1	0.0624	0.2195	NS	NS
Error	40	0.2843			
Total	53	0.3104			

* 0.05 and 0.01 refer to the significance level
# NS = not significant S = significant

TABLE IX

ANALYSIS OF VARIANCE OF DRY WEIGHTS OF STEMS

Source of Variation	Degrees of Freedom	Mean Square	F	Remarks* 0.05	0.01
Replication	8	1.5503	0.9179	NS	NS
Treatment	5	3.7435	2.2165	NS	NS
Error	40	1.6889			
Total	53	1.8618			

* 0.05 and 0.01 refer to the significance level.

TABLE X

ANALYSIS OF VARIANCE OF DRY WEIGHTS OF ROOTS

Source of Variation	Degrees of Freedom	Mean Square	F	Remarks* 0.05	0.01
Replication	8	0.6862	1.218	NS	NS
Treatment	5	0.1935	0.3436	NS	NS
Error	40	0.5632			
Total	53	0.5469			

* 0.05 and 0.01 refer to the significance level.

TABLE XI

ANALYSIS OF VARIANCE OF DRY WEIGHTS OF CAPSULES

Source of Variation	Degrees of Freedom	Mean Square	F	Remarks* 0.05	0.01
Replication	8	0.5054	0.1885	NS	NS
Treatment	5	2.5813	2.6773	S	NS
CGA vs. C	1	3.2427	3.2378	NS	NS
A vs. C	1	1.8560	1.8532	NS	NS
M vs. C	1	0.0288	0.0287	NS	NS
AGA vs. CGA	1	2.1632	2.1600	NS	NS
MGA vs. CGA	1	10.88889	10.8726	S	S
Error	40	1.0015			
Total	53	1.0851			

* 0.05 and 0.01 refer to the significance level.

Effect on Alkaloid Concentration

Effect on Leaves: The inhibitory effect on the alkaloid content of the leaves was not significant. There was a two percent increase in the A group as compared with the control, but there was a four percent decrease in the M group (Tables XII and XIII). From this it can be said that there was no significant residual effect of the inhibitors.

An 11 percent decrease compared with controls was noted in the CGA group. The AGA group showed a six percent increase over the A group and an eight percent increase over the C group. The MGA group demonstrated a two percent increase over the controls and an eight percent increase over the M group. From this it was concluded that the general effect of the GA-seed treatment was inhibitory to the controls, but stimulating to the plants previously treated with growth inhibitors.

Effect on Stems: The A group showed a seven percent increase over the controls, whereas the M group showed a 29 percent decrease as compared with the control. It was concluded that MH had a significant residual inhibitory effect, but the AMO 1618 did not.

The CGA plants showed a 15 percent decrease as compared

with the controls. The AGA group showed a seven percent decrease when compared to the A group, but it was equal in concentration to the control group. The MGA group showed a 14 percent increase over the M group. However, it still showed a 15 percent decrease from the control.

Effect on the Roots: The A and M groups showed 15 percent decreases from the controls.

The CGA and AGA groups had the same alkaloid concentration as the controls. A decrease of 15 percent was found in the A group compared with the AGA group. This indicated that GA-seed treatment overcame the inhibitory effect of AMO 1618 on alkaloid production. In the case of the MGA group the reversal of inhibition was even greater since this group was 110 percent of controls (Table XIII).

Effect on the Capsules. There was an inhibitory effect in both the A and M groups in comparison with the controls. The decreases in alkaloid concentration were nine percent in the case of the A group, and 22 percent in the case of the M group.

The CGA group showed no difference in the alkaloid content as compared with the controls. The AGA group did not differ from the A group; in other words the inhibition was not reversed. However, in the case of the MGA, the inhibition was significantly reversed. The alkaloid content was raised from 78 percent of

controls in the M group to 118 percent in the MGA group.

Conclusions from effect on alkaloidal concentration. It was concluded that the residual inhibition caused by AMO 1618 on the alkaloid concentration in the various plant parts was not marked. Likewise, the stimulating effect of the GA-seed treatments on these plants was not pronounced in the plant parts other than the leaves where there was a considerable increase in the AGA group (Figure 7). The residual inhibition of MH was more pronounced that that of the AMO 1618 and was quite marked in leaves and capsules. The GA treatment apparently reversed the residual inhibition.

TABLE XII

THE ALKALOID CONCENTRATION IN THE PLANT ORGANS

	Plant Parts			
Treatment	Leaves	Stems	Roots	Capsules
	(mg/Gm)			
C	3.36	0.56	0.56	2.84
CGA	3.00	0.48	0.56	2.83
A	3.44	0.60	0.48	2.60
AGA	3.64	0.56	0.56	2.60
M	2.82	0.40	0.48	2.24
MGA	3.46	0.48	0.62	3.36

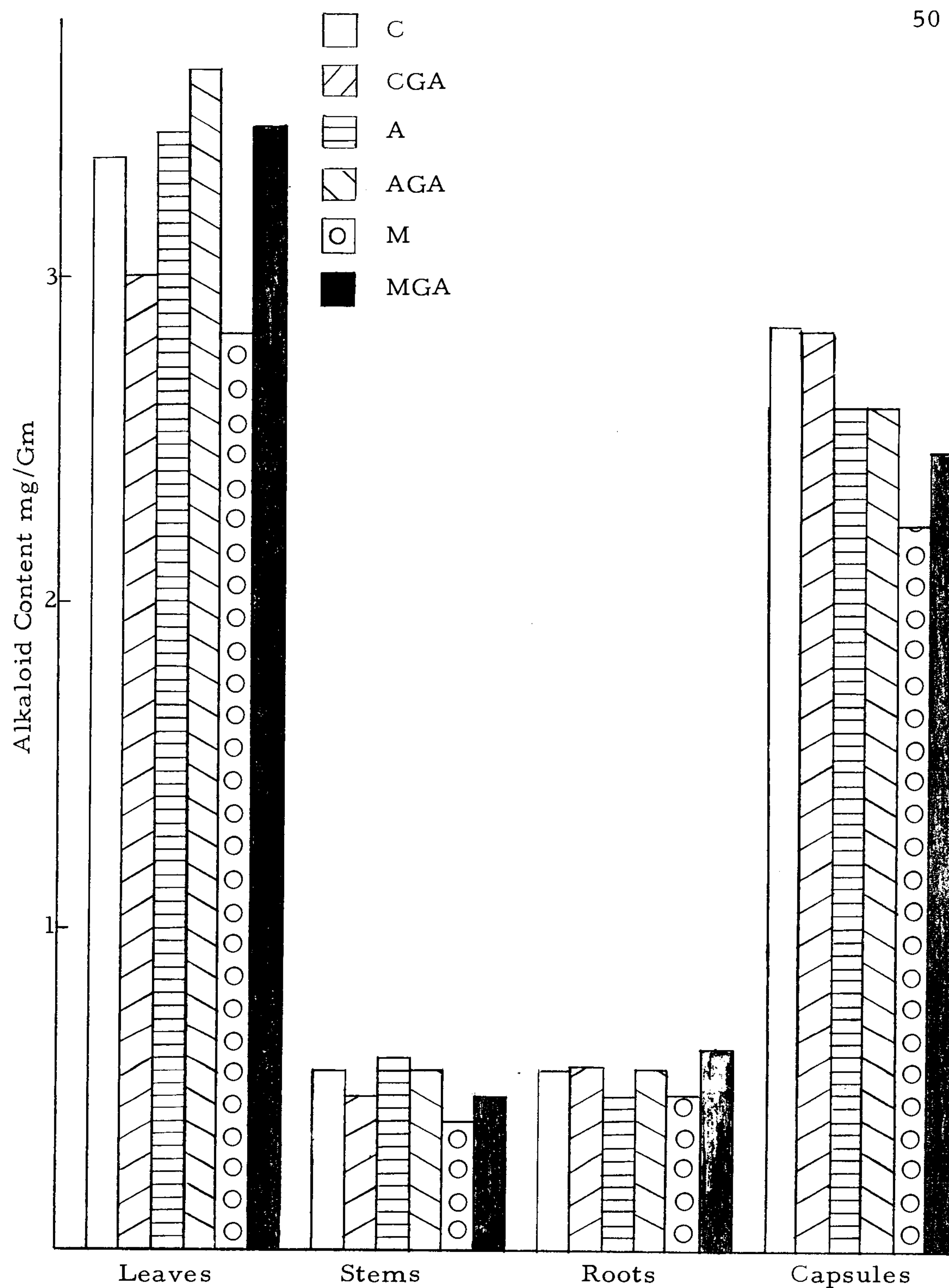


Figure 7. Effects of treatments on alkaloid concentration.

TABLE XIII

THE ALKALOID CONCENTRATION OF DIFFERENT TREATMENTS EXPRESSED AS PERCENT OF CONTROLS

Treatment	Plant Parts			
	Leaves	Stems	Roots	Capsules
	Percent			
C	100	100	100	100
CGA	89	85	100	100
A	102	107	85	91
AGA	108	100	100	91
M	96	71	85	78
MGA	102	85	110	118

Effect on Total Alkaloid Content

Effect on Alkaloid Content: The leaves of all treated groups contained less total alkaloids than the controls (Tables XIV-XV). However, the GA-seed treatment induced increased alkaloid production in the A and M groups. Total stem alkaloids were less in the treated groups than in the controls. The GA-seed treatment increased the alkaloid production in the stems of the M group only. Total root alkaloid was decreased in all the treated groups except for the AMO group which had 11 percent more alkaloids than the controls. Definite stimulation of alkaloid production was noted in both the A and M groups by the GA-seed treatment. A similar pattern was noted in the total alkaloids of the capsules and the whole plant (Tables XIV-XV).

TABLE XIV

TOTAL ALKALOID CONTENT* OF PLANT PARTS

Treatment	Plant Parts				
	Leaves	Stems	Roots	Capsules	Whole Plant#
			(in mg)		
C	20.79	7.71	1.54	10.53	40.57
CGA	17.58	6.87	1.52	8.20	34.14
A	18.95	7.70	1.34	7.87	35.86
AGA	19.65	7.58	1.52	8.68	37.43
M	16.63	5.70	1.24	7.92	31.49
MGA	19.96	7.08	1.71	14.54	43.29

* Calculated from dry weight and alkaloid analyses data.

Whole plant = leaves + stems + roots + capsules.

TABLE XV

TOTAL ALKALOID CONTENT AS PERCENT OF CONTROL

Treatment	Plant Parts				
	Leaves	Stems	Roots	Capsules	Whole Plant
			Percent		
C	100	100	100	100	100
CGA	84	89	98	77	84
A	91	99	87	74	88
AGA	94	98	98	82	92
M	80	73	80	75	77
MGA	96	91	111	138	106

Chlorophyll Data

In general, the control of chlorophyll a was higher than chlorophyll b(Table XVI). There were no significant differences between the various treatments on either the quantity or the ratio a/b. However, a considerable decrease in chlorophyll was noted in the MGA group.

TABLE XVI

CHLOROPHYLL CONTENT OF LEAVES OF PLANTS

Treatment	Chlorophyll a	Chlorophyll b
	(Calculated as mg/Gm)	
C	3.8	3.0
CGA	3.8	3.2
A	3.8	3.4
AGA	3.8	3.4
M	3.8	3.4
MGA	3.4	2.8

Data of Selective Solvent Extractions

The weight of the petroleum ether extract was considerably less in both the A and M groups than the controls (Figure 8, Table XVII). The amount of petroleum ether extract from the GA-seed treatment was less in the control and AMO 1618 groups, but considerably more in the MH group.

Significant increases were noted in the absolute ether extractives of the A, AGA, M, and MGA groups. The GA-seed treatment caused a considerable reduction of this fraction in the AMO 1618 group.

There were marked decreases compared with controls in the weights of the alcohol extract in the A and M groups.

The GA-seed treatment caused a marked decrease in the case of the control group, but, it induced considerable increases in the case of A and M groups.

There were slight decreases in the weights of the water extract in the A and M groups; these decreases were not as pronounced as that of the alcohol extract. The GA-seed treatment, in this case, caused a marked increase in the water extract of controls. There were no marked differences in the other groups due to the GA application.

Conclusion of selective solvent extraction: AMO 1618 and MH caused decreases in the principles extracted by petroleum ether, alcohol and water. However, a significant increase was noted in the ether extractive. GA-seed treatment reversed the decreases noted in the petroleum ether and alcohol extractives. The reversing effect was most pronounced in the case of alcohol. However, GA-seed treatment of control plants caused a decrease in the components.

soluble in alcohol. This suggests that GA-seed treatment has a stimulating effect primarily on plants previously treated with plant inhibitors.

TABLE XVII

WEIGHTS OF COMPONENTS EXTRACTED BY SELECTIVE SOLVENTS

Solvent Treatment	Petroleum Ether	Absolute Ether	95% Alcohol	Distilled Water
	(Calculated as mg/Gm)			
C	115.0	6.1	198.7	197.6
CGA	112.0	7.2	135.8	214.7
A	104.6	14.1	158.7	189.0
AGA	101.1	14.0	201.7	192.6
M	100.3	19.5	163.0	183.2
MGA	114.5	10.1	200.5	179.2

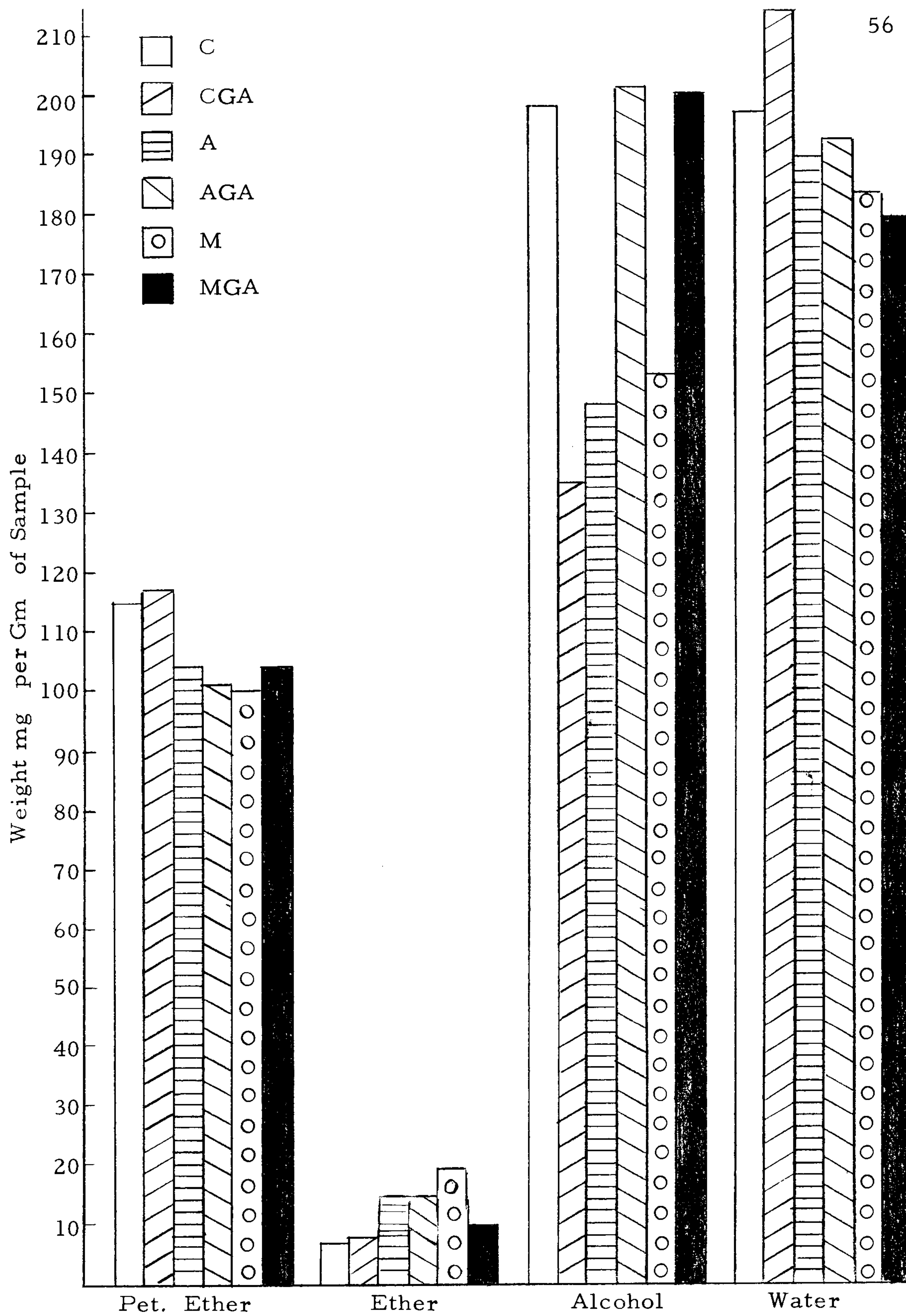


Figure 8. Results of selective solvent extraction.

V. SUMMARY AND CONCLUSIONS

Growth Patterns

No marked differences were obtained in growth patterns as determined by height measurements from the various treatments. It was concluded that the inhibitory effects of AMO 1618 and MH noted in the first generation plants did not manifest itself in the second generation. GA-seed treatment did not significantly affect the height of the plants.

Fresh Weights

No inhibition of growth as demonstrated from fresh weight data was noted in the second generation of plants treated the previous year with MH. Although variable degrees of inhibition were noted in the various organs of the AMO 1618 group, marked inhibition was noted on a per-plant basis.

GA-seed treatment decreased the fresh weight of the control group. However, it caused increases in the plants previously treated with AMO 1618. From this it was concluded that GA reversed the residual inhibitory effect on fresh weights caused by previous treatment with AMO 1618. There was no marked effect of GA-seed treatment on plants previously treated with MH.

Dry Weights

The analysis of variance of dry weights indicated that AMO 1618 caused a significant decrease in the dry weights of leaves of the second generation when compared with controls. The capsules of the MGA group showed a significant increase in weight over the CGA group. This indicates that the stimulating effect of GA is more pronounced on inhibited plants than on control plants.

Alkaloid Concentration

The residual inhibitory effect of AMO 1618 was not marked and was not consistent in all plant parts. The stimulating effect of GA on these plants, consequently, was not appreciable. In the case of MH there was considerable residual inhibition, especially in leaves and capsules. The inhibition was markedly reversed by GA-seed treatment.

Alkaloid Content

There was a residual decrease in alkaloid content of the whole plant and plant parts due to both inhibitors. The effect of the MH was more pronounced than that of the AMO 1618.

The application of GA to the seeds of the control plants caused

a decrease in alkaloid content, whereas it caused reversal of the residual inhibition caused by the growth retardents. This reversal was so pronounced in the case of the plants previously treated with MH that the MGA group exceeded the controls. This was the only treatment that showed increase over the controls.

Chlorophyll

There were no marked differences between the variously treated plants either in quality or quantity of chlorophyll.

Selective Solvent Extraction

The constituents that were soluble in petroleum ether, alcohol and water were decreased by the residual effect of both AMO 1618 and MH. This decrease was reversed by GA-seed treatment. However, GA-seed treatment on control plants caused a decrease in constituents soluble in alcohol. Two- to three-fold increases were noted in the ether soluble fractions of plants treated with the inhibitors.

BIBLIOGRAPHY

1. Ambrose, D. G. and L. A. Sciuchetti. Influence of Kinetin and GA on growth and alkaloid patterns in Datura meteloides. Journal of Pharmaceutical Sciences 51(10):934-938. October, 1962.

2. Andreae, W. A. and S. D. Andreae. Studies on indoleacetic acid metabolism. I. The effect of methyl umbelliferone, maleic hydrazide, and 2, 4-D on indoleactic acid oxidation. Canadian Journal of Botany 31:426-437. 1953.

3. Audus, L. G. Plant growth substances. London, Hill. 1953. 465p.

4. Baker, J. E. Action of maleic hydrazide on physiological processes of tobacco and other plants. Physiologia Plantarum 14:76-88. 1961.

5. Brian, P. W. and H. G. Hemming. The effect of maleic hydrazide on the growth response of plants to gibberellic acid. The Annals of Applied Biology, Cambridge 45:489-797. 1957.

6. Brian, P. W. Influence of gibberellins on plant growth and development. The Journal of the Royal Horticultural Society 85:167-173. 1960.

7. Brummett, R. E. and L. A. Sciuchetti. Changes induced by gibberellic acid on growth and alkaloid patterns in Datura Stramonium Linne' and in Atropa Belladonna Linne'. Journal of American Pharmaceutical Association Scientific Edition 49:247. 1960.

8. Bukovac, M. J., and S. H. Wittwer. Gibberellic acid and higher plants. I. General growth responses. Quarterly Bulletin of Michigan Agricultural Experimental Station 39:307-320. 1956.

9. Burk, L. G. and T. C. Tso. Effects of gibberellic acid on nicotiana plants. Nature 181:1672-1673. 1958.

10. Caldwell, E. L. and L. A. Sciuchetti. The effect of gibberellin and other treatments on the germination and subsequent biogenesis of alkaloids in Datura Stramonium Linne'. Journal of Pharmaceutical Sciences--Submitted for publication, 1963.

11. Cathey, H. M and N. W. Stewart. Comparative plant growth retarding activity of AMO 1618, Phosphon and CCC. The Botanical Gazette 123(1):51-56. 1961.

12. Cathey, H. M. Mutual antagonism of growth control of Chrysanthemum morifolium by gibberellin and AMO 1618. Plant Physiology 33(suppl):XIiii. 1958.

13. Crafts, A. S., H. B. Currier and H. R. Drever. Some studies on the herbicidal properties of maleic hydrazide. Hilgardia 27:723-757. 1958.

14. Curry, G. M. and E. C. Wassink. Photoperiodic and formative effects of various wavelength regimes in Hyoscyamus niger as influenced by gibberellic acid. Mededel Lanbouwhoogeschool Wageningen 56(14): 1. 1956.

15. Ermalaeva, E. Y . and N. A. Kalzlowa. Effect of maleic hydrazide on the rate of photosynthesis and on the carbohydrate metabolism of plants. Akademiia Nauk SSSR. Botaniehenkii Institute - Trudy, Seriia 4: Experimental Naia Botanica. No. 15:120-132. 1962. (Abstracted in Chemical Abstracts 57(12):15551 F. 1962)

16. Fish, F. Effects of gibberellic acid on the growth and alkaloid content of Datura Stramonium Linne'. Journal of Pharmacy and Pharmacology 12:428-436. 1960.

17. Gautheret, R. J. Recherches sur l'actionde l'hydrazide maleique et de l'acid indole-acetic sur les cultures de tissues de topinambour. Comptes Rendu Academies des Sciences 234:2218-2221. 1952.

18. Gjerstad, G. Metabolic and morphological changes induced by gibberellic acid on Mentha piperita Linne'. Planta Medica 8:127. June 1960.

19. Grenlach, V. A. and J. G. Haesloop. Some effects of maleic hydrazide on internode elongation, cell enlargement, and stem anatomy. American Journal of Botany 41: 44-50. 1954.

20. Hisatomi, A. and L. A. Sciuchetti. Unpublished research on the effects of AMO 1618 and maleic hydrazide on growth patterns in Datura tatula. Corvallis, Oregon State University, School of Pharmacy, 1961.

21. Hisatomi, A. and L. A. Sciuchetti. Unpublished research on the effects of AMO 1618 and maleic hydrazide on alkaloid biogenesis on Datura tatula. Corvallis, Oregon State University, School of Pharmacy, 1961.

22. Kapoor, L. D. and B. K. Kaul. Preliminary studies on the effect of gibberellic acid on the growth and total alkaloid patterns of Datura innoxia Mill. Planta Medica 10:72-76. 1962.

23. Kato, J. Studies on the physiological effect of gibberellin. II. On the interaction of gibberellin with auxins and growth inhibitors. Physiologia Plantarum 11:10-15. 1958.

24. Lang, A. Introduction of flower formation in biennial Hyoscyamus by treatment with gibberellin. Die Naturwissenschaften 43:284-285. 1956.

25. Leopold, A. C. and W. H. Klein. Maleic hydrazide as an antiauxin in plants. Science 114:9-10. 1951.

26. Lockhart, J. A. Kinetic studies of certain antigibberellins. Plant Physiology 37(6):759-764. November, 1962.

27. Marth, P. C. and J. W. Mitchell. Plant growth suppressants with special reference to persistence of AMO 1618 in soil. Proceeding of American Society of Horticultural Science 76:673-678. 1960.

28. Masuda, J. Y. and G. H. Hamor. A note on the effects of gibberellins on alkaloidal content of Hyoscyamus Niger. Journal of American Pharmaceutical Association, Scientific Edition 48:361. 1959.

29. Naylor, A. W. and E. A. Davis. Maleic hydrazide as a plant growth inhibitor. Botanical Gazette 112:112-126. 1950.

30. Parups, E. Influence of gibberellic acid on the nicotine content of cigar tobacco. Canadian Journal of Plant Science 39:48-55. 1959.

31. Ragan, N. L. and L. A. Sciuchetti. Effect of gibberellic acid on the second generation of Datura Stramonium Linne'. Journal of Pharmaceutical Sciences 50(1): 87-88. January 1961.

32. Rainbow, Color and Chemical Company, Inc. Northridge, California. Personal communication.

33. Rosenthaler, Leopold. The chemical investigation of plants. London, Bell, 1930. 197p.

34. Sachs, R. M. and L. Lang, Shoot histogenesis and the subapical meristem: The action of gibberellic acid, AMO 1618 and maleic hydrazide. In: Plant growth regulation. Fourth international conference on plant growth regulation. Ames, Iowa State University Press, 1961. 567-577.

35. Schoene, D. L. and O. L. Hoffman. Maleic hydrazide, a unique growth regulant. Science 109:588-590. 1949.

36. Sciuchetti, L. A. The effect of gibberellic acid on the second-year growth and alkaloid formation in Atropa belladonna Linne'. Journal of the American Pharmaceutical Association. Scientific Edition 48(9):490-499. September 1959.

37. Sciuchetti, L. A. Gibberellic acid, its influence on medicinal plants. Part 3. Pacific Drug Review 72(8):16. 1960.

38. Sciuchetti, L. A. Influence of gibberellic acid on medicinal plants. Journal of Pharmaceutical Sciences 50(12): 981-997. December 1961.

39. Smith, G. M. and L. A. Sciuchetti. The influence of gibberellic acid on the growth and alkaloid biogenesis in Datura stramonium Linne' and Atropa belladonna Linne'. Journal of the American Pharmaceutical Association, Scientific Edition 48(1):63-68. January 1959.

40. Stewart, N. W. and H. M. Cathey. Applied aspects of the gibberellin. Annual Review of Plant Physiology 12:369-377. 1961.

41. Stowe , B. B. and T. Yamaki. Gibberellins: Stimulants of plant growth. Science 129:807-816. 1959.

42. Strudemeyer, B. E. The effect of maleic hydrazide on the anatomical structure of croff easter lillies. American Journal of Botany 40:25-29. 1953.

43. Wittwer, S. R. and M. J. Bukovac. The effect of gibberellin on economic crops. Economic Botany 12:213-255. 1958.

44. Zukel, J. W. A literature summary on maleic hydrazide. MIHS No. 8. Naugatuck, Connecticut, Naugatuck Chemical Division, United States Rubber Co., 1957. lp.