INTERRELATION OF RESPONSE TO INDOLE ACETIC ACID, DURATION AND INTENSITY OF LIGHT, AND HETEROSIS IN THE SNAPDRAGON (*ANTIRRHINUM MAJUS* L.)

By

JOHN BERNARD GARTNER

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Departments of Horticulture, Botany and Plant Pathology

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The author is greatly indebted to his wife who helped in the conducting of the experiments and compilation of data and typing. Without the inspiration of my wife, this work would never have been completed, and to whom the results are herewith dedicated.

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AN ABSTRACT

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APPROVED Charles L. Hamner

John B. Gartner

It was found that Antirrhinum majus L. definitely exhibits heterosis. An interrelationship was found between indole acetic acid, light intensity and the degree of heterosis. The degree of heterosis exhibited was not the same at all seasons of the year. Heterosis was measured by taking the increase of the F_1 over the average of the two parents both as to increase in height and increase in dry weight.

The degree of heterosis exhibited in A. majus is correlated with solar radiation. When solar radiation was low the degree of heterosis was not as pronounced as when solar radiation was high.

Treatment with indole acetic acid indicated that the hybrids showed a greater epinastic response than either of the parents. This epinastic response was also correlated with solar radiation and a greater epinastic response was found under low light intensity than under high light intensity. By using a biological test it was found that the inbreds have a more effective mechanism of inactivating or inhibiting the effects of indole acetic acid than the hybrid. It was found that solar radiation influences heterosis in A. majus. The expression of heterosis in A. majus can be directly correlated with light intensity and as the light intensity increases the degree of heterosis increases.

The inbreds possess a mechanism of inactivating indole acetic acid. This inactivation increases as light intensity increases and this possibly could be the explanation of heterosis in A. majus. There is less inactivation of growth substances in the hybrids than in the inbreds under high light intensities. This results in an increased growth rate in the hybrids and could be the reason why the hybrids show a greater amount of heterosis under high light than under low light intensities.

John Bernard Gartner

Candidate for the degree of

Doctor of Philosophy

Final examination, July 7, 1952, 2:00 P. M., Horticulture Seminar Room.

Dissertation: Interrelation of Response to Indole Acetic Acid, Duration and Intensity of Light, and Heterosis in the Snapdragon (Antirrhinum Majus L.)

Outline of Studies

Major subjects: Horticulture and Botany

Biographical Items

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TABLE OF CONTENTS

INTRODUCTION

The superior vigor of the progeny of two unrelated inbred lines has been observed and utilized for many years. This phenomenon has found more applications continously from pre-Mendelian times to the present and additional applications will undoubtedly be made in the future.

The term hybrid vigor originally applied to this phenomenon has fallen into some disrepute. It has been used in cases where the parents were not inbred, applied to generations later than the F_1, and came to imply a consistent superiority which is not necessarily the case. Shull (39) proposed the term heterosis to describe the vigor difference, positive or negative, between the F_1 and the parental average.

Most of the work on heterosis has been done on corn. The American Indians knew without experimental evidence that planting of mixtures of corn would increase the yield. However, corn is not the only crop that shows heterosis.

The increased yield of hybrid over inbred corn is very considerable. Greater yields are obtained with less effort. The use of hybrid corn, gave a surplus for the United States during the war and post war distress periods. This was great enough to help feed, not only the United States, but other countries as well. It would have taken a great deal more manpower to produce these same yields if it were not for heterosis. The value of hybrid corn to the Agricultural industry annually will more than pay for all the research work that has ever been done in the field of Agriculture. The new applications of heterosis offer greater promise for the future than have been realized in the past. Other plants such as onions tomatoes, etc., as well as many ornamental crops, show marked increase in growth of hybrids over inbreds.

For this investigation of heterosis, the snapdragon, _Antirrhinum majus_ was chosen as the test plant. Heterosis in this crop is not recorded in the scientific literature although its effect is attested by the rapid increase in the popularity of F_1 hybrids in commercial Floriculture.

Any new information on heterosis in this crop offers immediate advantage. Culturally, snapdragons are advantageous because large populations may be grown in comparatively small space and short time at any season of the year. Genetically it is diploid having sixteen somatic chromosomes and a considerable body of literature shows expected inheritance of unit factors, Bauer (6). It is self fertile, easily emasculated and pollinated, and produces as many as 500 seeds from a single pollination. It appears that no other plant offers all of these advantages for this type of work.

Inbred and hybrid snapdragons differ in their regional adaptation. In the author's experience outstanding hybrids from more northern latitudes have not been advantageous in North Carolina. Since these differences were known to exist the investigation of this crop offered promise of useful information.

REVIEW OF LITERATURE

It is commonly known to commercial growers that hybrids are much superior to inbreds in several species of shrubs and evergreens. This hybrid advantage is maintained by asexual propagation.

A search of the literature revealed little scientific information concerning heterosis in snapdragons. Post (35) has recommended the use of hybrid snapdragons. Since little is known concerning heterosis of Antirrhinum, a search of the literature was made to determine what was known on other plants.

Early plant hybridists including Kolreuter (27) in the eighteenth century, Gartner (18) in the nineteenth century noted increased vigor of hybrids. Since this time considerable experimentation has been carried out to find a basis for heterosis. Even today after many years research, no one has completely explained the exact mechanism. Since 1900 some reasonably satisfactory hypothesis of heterosis have been presented. The hypothesis that is most favored is the one of East and Jones (12, 13, 14, 15, 16, 22, 23, 24) involving dominance.

Jones (22) explanation of heterosis involves dominance well exemplified in Pisum sativum L. Keeble and

Pellew (26) have shown that two short plants crossed gave uniformly tall progeny. Genetic analysis has shown that one parent contributed a recessive factor for short internodes and a dominant factor for many nodes while the other parent contributed the alleles dominant long internodes and recessive few nodes. The F_1 showing the dominant alleles produces tall plants having many long internodes.

Crops where heterosis is important do not produce in F_2 and later generations the homozygous dominant size factors that might be expected from F_1 performance. As Jones (25) indicated it is almost impossible by studying the genetics of heterosis to obtain the expected genetic ratio in the F_2.

When Morgan (34) first discovered linkage, Jones (25) used this fact to show why the F_2 would not give the expected proportion of vigorous individuals. If the gene for the long internodes was closely coupled with the gene for a small number of nodes on one chromosome, and the allele for a large number of nodes was closely coupled with the allele for short internodes on the homologous chromosome these factors would be repulsion linked and would give a low rate of crossing over. The proportion of vigorous individuals would be reduced and the probability of the homozygous dominant recombination would be the square of the frequency of the low rate of crossing over.

Singleton (40) has calculated that 2000 times the land area of the earth would be necessary to grow enough corn plants to stand a reasonable chance of recovering a completely homozygous dominant, if thirty genes were involved in heterosis without linkage.

Since heterosis usually appears to be dependent on multiple factors and involves linkage, it is almost impossible to recover a homozygous plant in the F_2 or later generations that equals the F_1 in vigor. It has been shown by East (15) that the segregating population continually reduces in vigor each year until it reaches a static position when it is effectively homozygous. By continual inbreeding, especially with single plant selections, the yield will be reduced until effective homozygosity is obtained. However, complete homozygosity is very seldom obtained and East (13) has shown that after eight generations, ninety-five per cent homozygosity would be approached.

It is very difficult to determine what factors control heterosis, since it is difficult to measure growth. To date, there are comparatively few methods of determining rates of growth of one type or another because it is difficult to control so many of the influencing external environmental factors which play an important role in the response of a crop to its total inherent vigor.

The effect of heterosis in plants can not always be measured by an increase in size of the plant. It may be evident as an increase in yield or an increase in dry weight or an increase in nutritional value. For example, tomato hybrids do not necessarily show an increase in size of fruit, but may greatly increase the number of inflorescence as well as the total yield of fruit.

Whaley (50, 51) suggested that hybrid vigor is due to heterozygosity, and the more heterozygous the individual the more vigorous the individual. In some ways this seems to fit into the picture and could possibly explain why the F_2 and succeeding generations gradually decrease in vigor until homozygosity is obtained. Examples have been reported by Whaley (51) in which the further apart the parents in relationship, the more vigorous the hybrids. However, East (12, 13) has shown by inbreeding sister single plant selections of a segregating population to effective homozygosity and then crossing, that heterosis was evident although the lines were closely related. This is the basis for Shull (39) coining the term heterosis meaning that the individual was extremely heterozygous.

Prior to 1930, dominance and linkage were fairly well accepted as a basis of heterosis. Ashby (2, 3, 4, 5) was one of the first to discuss heterosis as related to growth factors.

From his investigations, he assumed that hybrid corn had approximately the same growth rate as its less vigorous parents, and that the hybrid did not vary from either of its parents as to cell size, photosynthetic activity or respiration. As a result, the flattening of its growth curve during the grand period of growth would be the same in inbreds as in hybrids . He concluded that the only advantage the hybrid had over its less vigorous parents was a larger embryo having a higher percentage of germination, and that this advantage was maintained throughout the grand period of growth and resulted in a superior plant.

Lindstrom (29, 30, 31) similarly came to the conclusion that the hybrids maintained an initial advantage in size over the inbreds. He stated that a small initial advantage in size in the F_1 cells or embryo would multiply very rapidly and work on the compound interest law and could possibly account for the superiority of the hybrids. Lindstrom (31) also decapitated some hybrids to reduce any initial size advantage and showed that they finally exceeded the parental strains as to dry weight. However, he supposed that this procedure did not entirely eliminate the embryonic capital and initial advantage, and he claimed that the meristems maintained this advantage. Further work along this line has failed to prove that the embryo is any larger in the hybrids than in the inbreds, and in some cases the hybrids were found to be intermediate in size, yet they still displayed more vigor than either of the parents.

East (15) pointed out that there are several recorded crosses where hybrid seeds were not as large as those of the larger parents. Most of these are in the leguminosae where there is virtually no endosperm. Hence, it would appear that the embryo size alone cannot be the important factor in hybrid vigor.

In the past few years, there has been considerable speculation on the one-gene one-enzyme theory on controlling plant growth.

Tatum (43) has shown that eye color in Drosophila is a chemical action and is gene-controlled.

Haney (21) has shown a sequence of gene action in color inheritance in snapdragon. The dominant allele for white must be present for expression of any color other than white. The dominant alleles for both white and ivory must be present to show anthacyanin pigmentation or its modification by dilution or intensity genes.

All the most recent work on physiolgical genetics has demonstrated that chemical processes involving enzymes are gene-controlled. Tatum and Beadle (7, 44) have shown that mutant strains of Neurospora are unable to snythesize certain essential growth substances. Such mutants could be obtained by treating normal strains with mutagenic agents.

When these mutant strains of Neurospora are supplied with the deficient growth substances, they are able to make as much growth as the normal strains of fungi. Work of this nature has indicated that heterosis may be the result of more efficient production of growth substances. This explanation would not disprove any of the previous theories but would confirm these concepts of heterosis.

Robbins (36) has shown that extracts of partially germinated inbred and hybrid corn, differed significantly as media for the growth of certain selected strains of fungi. The ones growing in the hybrid extract were much superior to those grown on inbred extracts. This may indicate that hybrids have a more efficient production of growth substances than their less vigorous inbred parents.

Whaley and Long (52) have shown that under certain conditions excised roots of hybrid tomatoes and hybrid corn grown in cultures show considerably more growth than those of their inbred parents. Robbins (37) using a cross of inbred lines of _Lycopersicum pimpinellifolium_ and _Lycopersicum esculentum,_ found in solutions supplemented by thiamine, pyridoxine, or nicotinamide, separately or in combinations of two, that the F_1 roots grew more rapidly and produced more dry material than those of the inbred parents. He showed that the roots of Parent A showed a greater response to pyridoxine than Parent B. The roots of Parent B showed a greater response to nicotinamide than those of Parent A. However, Parent B approached the F_1 in solution containing all three supplements.

Whaley (51, 53) has found in inbred lines of maize, of the varieties Pipe, Pawnee, and their reciprocal hybrids, marked differences in their reactions to light conditions. With certain light intensities, the hybrids showed a superiority and at other intensities the inbreds approached the hybrids in size.

Malinowsky (32, 33) noted that heterosis in beans produced a response similar to that described by Whaley (51) in maize under certain environmental conditions.

Goldschmidt (19, 20) has shown that genes influence enzyme reactions. This is further controlled by environment. Phenocopies of moths may be obtained by changing the external environment. Changed environment resulted in a duplication of the wing pattern of another genetic strain of moth. These phenocopies show that under different environmental conditions, the course or sequence of chemical reactions may be altered and as a result gene action will be modified.

Van Overbeek (46) has shown that "Laziness" in maize is due to an abnormal distribution and production of growth substances.

Galston (17) has shown that riboflavin in the presence of light inactivates indole acetic acid. It also can be demonstrated that under varying intensities and qualities of light indole acetic is inactivated.

Wittwer (54, 55) has shown that in the use of indole acetic acid for fruit set, greater efficiency was obtained at low than at high light intensities.

Skoog (41, 42) has found that adenine in the present of light will inactivate indole acetic acid. A good example of this is the original work on phototropism.

Burkholder and Johnston (9), Boysen Jensen (8), Went (49) and other showed that light inactivates the growth substance on the light side of the coleoptile resulting in its bending towards the light. It has been observed for many years by many botanists, that plants grown in the Alpine areas are dwarfed, Went and Thimann (49). A possible explanation is that they receive greater quantities of ultra violet light which is known to inactivate the auxin, indole acetic acid, and hence result in less growth.

It is appearent that while the chemical reaction of plants may be gene-controlled, environmental factors may alter its mode of action.

MATERIALS AND METHODS

A. Plants

Two inbred lines of *Antirrhinum majus* L. and the hybrid produced from the cross between these two inbreds were chosen as experimental test subjects for this project. *A. majus* was chosen since this species displays heterosis when two inbred lines are crossed; also the genetics of this species is fairly well known. By using *A. majus* as a test subject, a large population can be grown in a comparatively small amount of space. By planting three inches by three inches in a greenhouse bench, four feet by ninety feet, it was possible to raise 2262 plants. This gives an adequate population for analysis in a comparatively small space. A complete crop rotation can be made within the period of ten weeks and hence it was possible to obtain considerable data. The plants were grown under greenhouse conditions during the period from September 1951 to May 1952 with temperature fluctuations of 50° to 70° F.

The seedlings were germinated in a constant water level bench and transplanted as soon as possible to maintain uniformity in the individual plots.

Seedlings were planted in a greenhouse bench containing a soil mixture of a silt loam with manure and peat added. Nutrient levels were maintained by liquid feeding whenever the soil tests indicated a low level of nutrients.

At this spacing competition between plants would be intense in later stages of growth, measurements were taken when the F_1 reached ten centimeters in height. Otherwise environmental variation within plots as well as between plots would be so intensified to such a degree as to greatly increase the experimental error.

The following lines were selected for these experiments. P_1 is a strain of Rockwood's Pink Supreme originally secured from the George J. Ball Company, West Chicago, Illinois. P_2 is an inbred isolated from the variety Helen Tobin from the introduction of Dr. Harold White of the University of Massachusetts.

These inbred lines are single plant selections from these strains and have been inbred for the last five generations from what were apparently true lines. P_1 is an inbred that develops slowly but has a large production of dry weight and is a very sturdy plant. P_2 develops rapidly but does not have a large amount of dry weight in relationship to its size.

The F_1 of this cross is a very vigorous growing hybrid. It was outstanding in this respect in a survey of 350 F_1 snapdragons.

B. Measurements of Heterosis

To obtain experimental evidence of heterosis in *A. majus* three experiments were conducted to obtain data on height and dry weight of the parents in the F_1.

Experiment I was conducted by sowing seed December 1, 1951 and on January 15, 1952 these plants were benched. Height measurements were taken on February 5, 1952.

Experiment II - seeds were sown on February 3, 1952 and benched on March 28, 1952. Measurements of height were taken on April 6, 1952.

Experiment III - Seeds were sown on March 28, 1952 and on April 19th these plants were benched. Height measurements were taken on May 8, 1952.

The same procedure was followed in Experiments I, II, and III. Seeds of P_1, P_2, F_1 and F_2 were sown on the respective dates and in each experiment, 350 plants of P_1, P_2, and F_1 were benched before the seedlings became crowded in the propagation bench. Along with the hybrid and the two parents, 1250 plants of the F_2 segregating population were benched at the same time and selected at random in order to avoid distorting the curve of segregation. Measurements were taken when the hybrid was approximately ten centimeters in height. This size was chosen in order to avoid effects of overcrowding.

C. Dry Weight Determinations

Determination of dry weights of the P_1, P_2 and F_1 plants were made after height measurements and response to indole acetic acid was recorded. Fresh weights were taken and then the plants were placed in an oven at 100° C. and then dried for a period of 72 hours.

D. Response to Indole Acetic Acid

The plants in Experiments I, II, and III were sprayed with indole acetic acid after height measurements were recorded. Plants in Experiment I were sprayed at 100 ppm. and exhibited typical epinasty. However, it was found that plants grown in Experiments II and III during which the light intensities were high and a stronger concentration of indole acetic acid was needed to produce the same degree of response. A concentration of 1000 ppm. of indole acetic acid was used in these experiments.

A hand atomizer was used in applying this chemical spray with each lot receiving the equivalent volume of material at the same concentration. One-half of the plants were left untreated and were used as controls. The response of the plants to the indole acetic acid was determined by an arbitrary standard using the numbers 0-5 to designate the degree of epinasty with the higher number showing the greatest epinasty and the lowest number showing no epinasty. The degree of epinasty was recorded as soon as there was a difference between the inbreds and the hybrids.

In order to determine if a correlation existed between height and epinastic response plants were recorded and identified in such a manner that a comparison of height and epinasty could be made for each individual plant.

E. Biological Tests

To determine the presence of an inhibitor or an inactivator in the inbreds a biological test was employed. Alamercery (1) has shown that cucumber seedlings are very sensitive to indole acetic acid in their response to germination and growth of hypocotyl and roots. When seeds are grown in a solution of indole acetic acid, the amount of root growth inhibition is in proportion to the concentration of indole acetic acid. Hence, this method was used to determine the presence of an inhibitor or inactivator of indole acetic acid.

In order to determine whether or not an inhibitor or an inactivator was present, ten grams of plant material from the tops of P_1, P_2, and F_1 were macerated in a waring blendor and made up to a volume of 100 cc. by using distilled water. To this slurry, indole acetic acid was added to make a final concentration of 25 ppm. Sixteen cucumber seedlings were placed on a filter paper in a petri dish, and five cubic centimeters of this slurry was added to each petri dish. In addition a distilled water solution of indole acetic acid at 25 ppm. and distilled water alone were used as controls. Each treatment was replicated three times. The cucumber seedlings were allowed to germinate under a controlled temperature, both in constant light and in darkness. Approximately four days after treatment root measurements in millimeters were taken as well as percentage of germination. Since a significant difference was found the localization of the inactivator was attempted.

In an attempt to localize the active principle plants were divided into two lots before being macerated in a waring blendor. Meristematic tissue was separated from older tissue and the samples were prepared separately and made up to equivalent concentrations.

F. Effect of Light Intensity

To determine the effect of light intensity on the response of snapdragons to indole acetic acid, 1200 plants each of F_1, P_1, and P_2 were used. One-half of these plants were grown in an open bench receiving full light and one-half were grown under a cheesecloth shade. Krone and Bulmer (28) have shown that cheesecloth shade reduces solar radiation by approximately 45%. In each plot 400 plants were treated with indole acetic acid after growing under these conditions for a period of three weeks. After treatment one-half of the treated plants in each plot were shifted to the other environment.

To determine the effect of light intensity on the growth of inbreds and hybrid a control plot of P_1, P_2, and F_1 containing 200 plants each were grown under each of the above light conditions. The control plots remained under the original environmental conditions throughout the experiment.

G. Solar Radiation

Solar radiation measurements were obtained from the United States Department of Agriculture Hydrologic Station at Michigan State College, East Lansing, Michigan. Measurements were in gram calories per square centimeter. Crabb (10, 11) shows, gram calories per square centimeter is directly proportional to foot candles as a measurement of light intensity.

RESULTS

I. Heterosis

In Experiment I heterosis was shown to exist when *A. majus* was grown from December 1, 1951 through February 5, 1952. (Figure I). The hybrid did not surpass the P_2 but did surpass P_1. The hybrid was .73 centimeters superior in height to the mid-parent mean. (Table 1).

The hybrid shows a superiority in dry weight over either of the parents and an increase of .115 grams over the mid-parent mean. (Table 2). The segregating population (F_2) as shown in Table 1 has a wide range of vigor; some of the plants being smaller than either of the parents but none of them surpassing the hybrid. This is what is normally expected in a segregating F_2 population. Heterosis was still displayed due to the fact that the hybrid was superior to the mid-parent mean.

An increase in the amount of heterosis as shown in Figure II and Table 2 was found in Experiment II. The hybrid surpassed both of the parents in height and in dry weight having an increase over the mid-parent mean of 3.65 centimeters per plant, and .354 grams per plant.

TABLE 1

Average height in centimeters of 350 plants each of inbred (P_1 and P_2) and the hybrid (F_1) and segregating population (F_2) of *A. majus*.

	Parent 1 (P_1)	Parent 2 (P_2)	Mid-parent mean	Hybrid (F_1)	(F_2)	Heterosis*
Exp I **	7.3	12	9.65	10.38	7.91	0.73
Exp II***	5.9	8.93	7.415	11.1	9.9	3.65
Exp III****	4.7	9.2	6.95	10.9	6.9	3.95

* Heterosis is the difference between mid-parent mean and F_1.

** December 1, 1951 through February 5, 1952.

*** February 3, 1952 through April 6, 1952.

****March 28, 1952 through May 8, 1952.

TABLE 2

Average dry weight in grams of individual plants of Parent 1 (P_1) Parent 2 (P_2) and hybrid (F_1). Individual averages are based on the average of 350 plants.

	Parent 1 (P_1)	Parent 2 (P_2)	Mid-parent mean	Hybrid (F_1)	Heterosis*
Exp I**	0.216	0.357	0.286	0.401	0.115
Exp II***	0.231	0.206	0.216	0.570	0.354
Exp III****	0.12	0.16	0.14	0.6	0.46

* Heterosis is the difference of the F_1 from the mid-parent mean.

** December 1, 1951 through February 5, 1952.

*** February 3, 1952 through April 6, 1952.

**** March 28, 1952 through May 8, 1952.

Figure I

Comparison of height and frequency of P_1, P_2, F_1 and F_2 illustrating heterosis in *A. majus* with the normal segregation in the F_2.

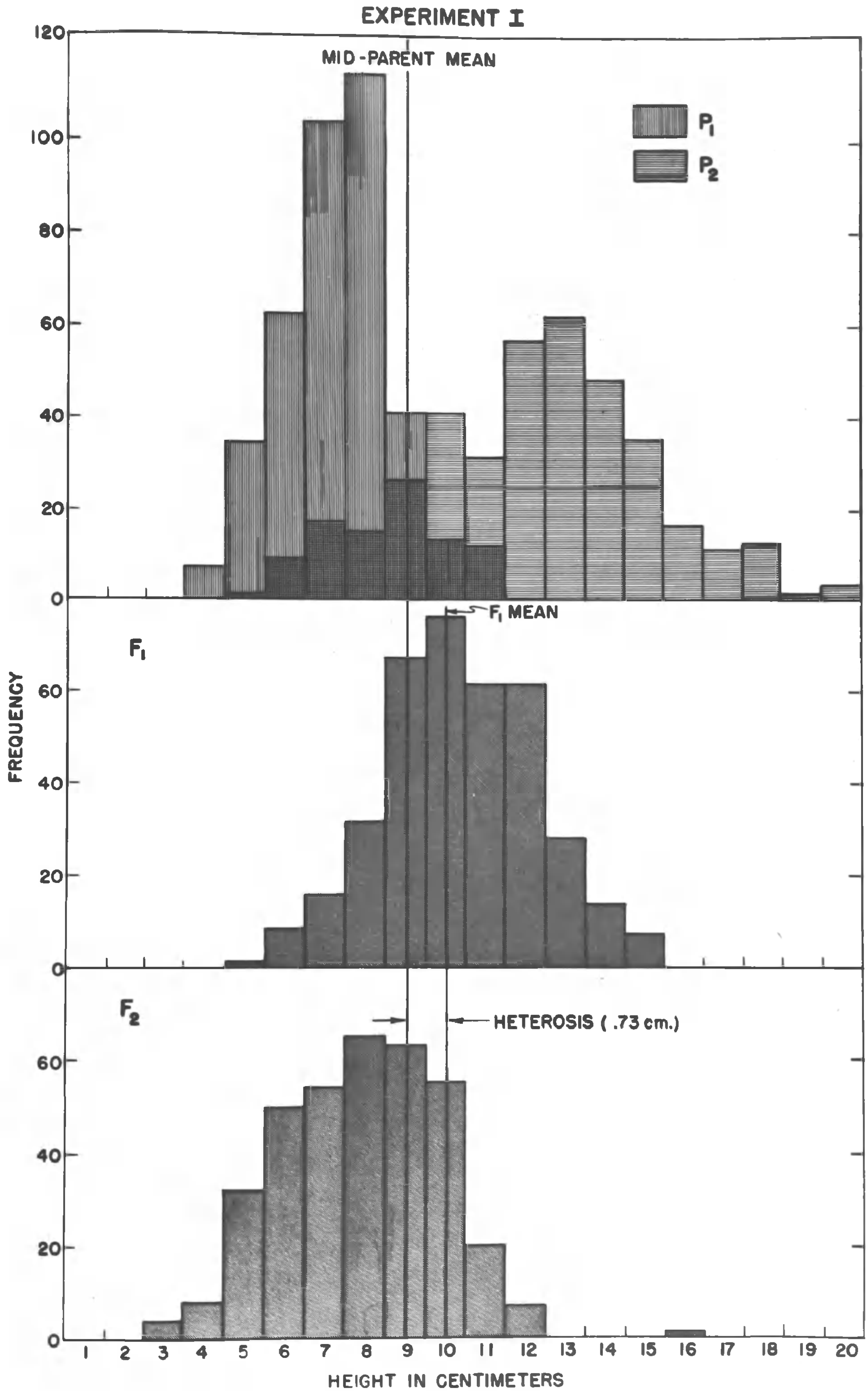
EXPERIMENT I
MID-PARENT MEAN
P1
P2
F1 MEAN
F1
F2
HETEROSIS (.73 cm.)
FREQUENCY
HEIGHT IN CENTIMETERS
0
20
40
60
80
100
120
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure I

Figure II

Comparison of height and frequency of P_1, P_2, F_1 and F_2 illustrating heterosis in *A. majus* with the normal segregation in the F_2.

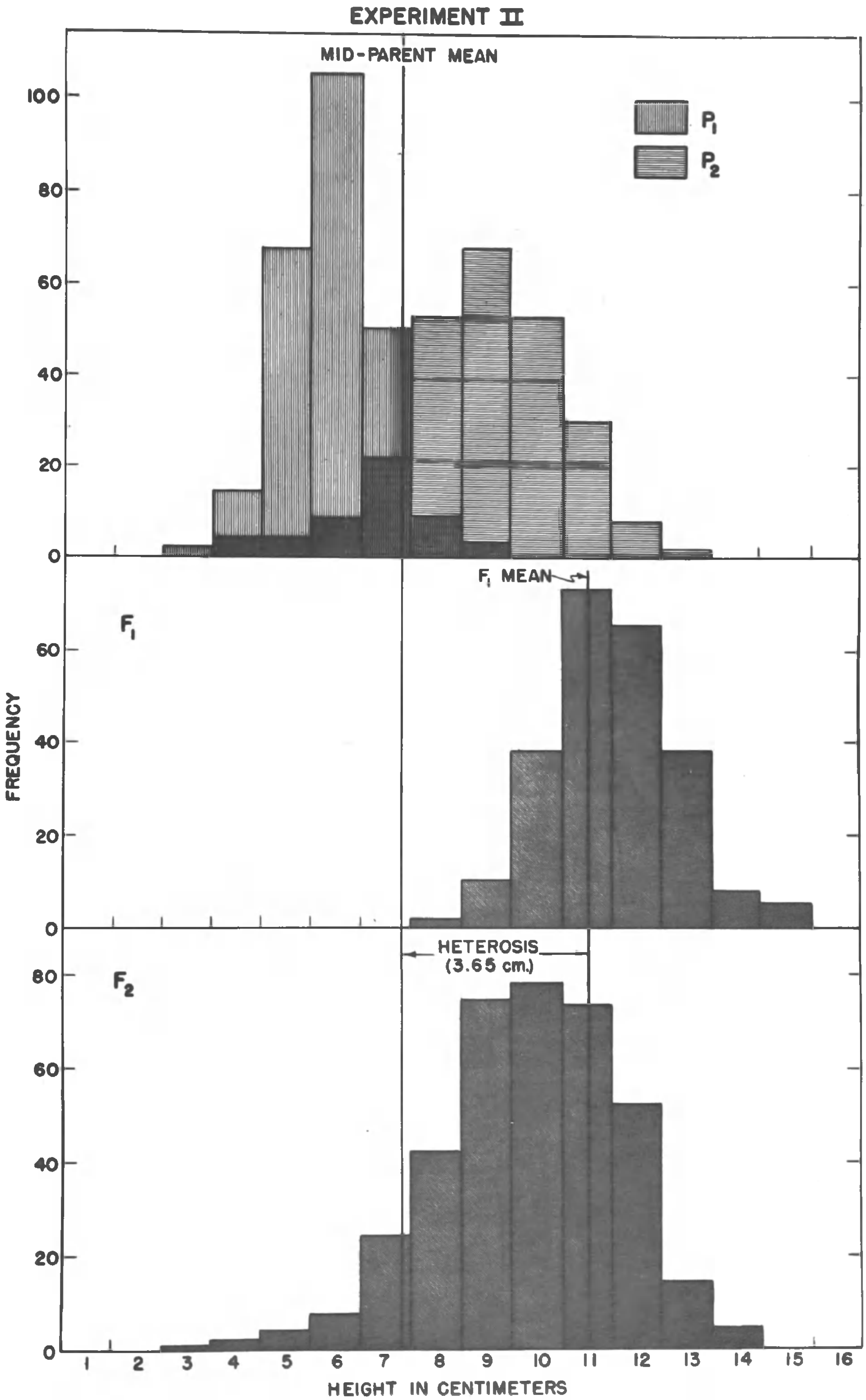
EXPERIMENT II
MID-PARENT MEAN
P1
P2
F1 MEAN
F1
HETEROSIS
(3.65 cm.)
F2
FREQUENCY
HEIGHT IN CENTIMETERS

Figure II

The F_2 again showed a wide range of vigor with some of the population being less vigorous than either of the parents and with none of the individuals surpassing the hybrid. The heterosis in Experiment II both as to height and dry weight is greater than in Experiment I.

Heterosis was again displayed with the hybrid surpassing both of the parents in Experiment III. As shown in Figure III and Tables 1 and 2 the increase in height per plant of the hybrids over the mid-parent mean was 3.95 centimeters. The increase in dry weight per plant of the hybrids over mid-parent mean was .46 grams. Experiment III showed a considerable increase of heterosis by both criteria over the two preceding experiments. An illustration of the difference between P_1, P_2 and F_1 can be seen in Figures IV and V.

II. Physiological Response

A. Physiological Response to Indole Acetic Acid

It was found in preliminary experiments that the hybrid exhibited an epinastic response to concentrations of indole acetic acid as low as 5 ppm. The parents exhibited little or no response at this concentration. Further investigations proved that the hybrids exhibited a greater response to indole acetic acid than either of the parents. (Figure VI).

Figure III

Comparison of height and frequency of P_1, P_2, F_1 and F_2 illustrating heterosis in *A. majus* with the normal segregation in the F_2.

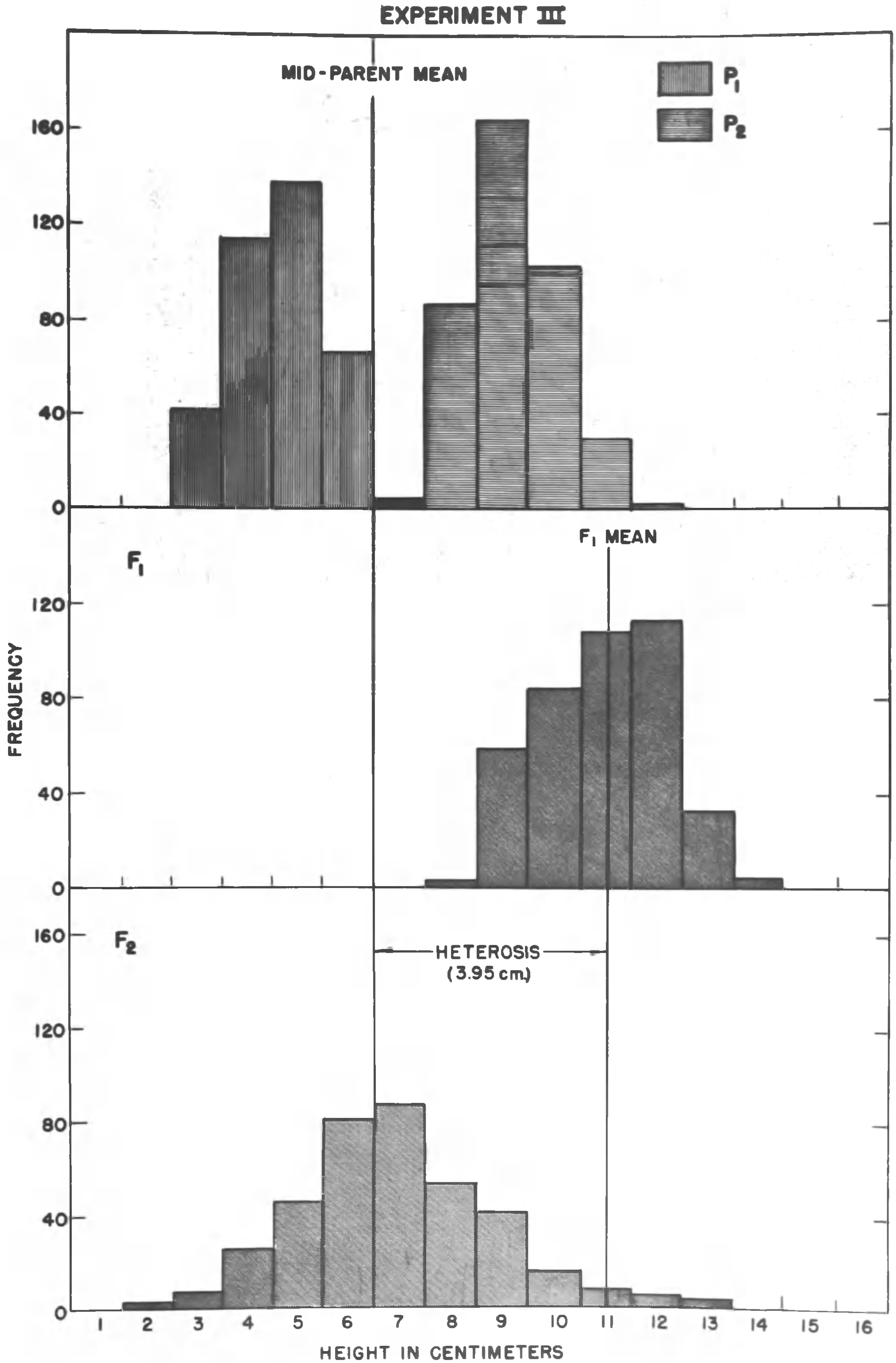
EXPERIMENT III
MID-PARENT MEAN
P_1
P_2
F_1
F_1 MEAN
F_2
HETEROSIS
(3.95 cm.)
FREQUENCY
0
40
80
120
160
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
HEIGHT IN CENTIMETERS

Figure III

Figure IV

A view of cultures showing uniformity of F_1 in foreground, P_1 center, P_2 background.

Figure V

The effect of Heterosis on the growth of *A. majus*. The plants reading from left to right are P_1, P_2, and the hybrid F_1.

This phenomena was more pronounced in the fall and winter than in the spring as is evident by the fact that the concentrations had to be increased in Experiments II and III from 100 ppm. to 1000 ppm. to obtain the same response as in Experiment I. Immediately after treatment with 1000 ppm. indole acetic acid, both the hybrid and the inbreds showed the same response. The inbreds recovered from this epinasty more rapidly than the hybrid. Epinastic response was evaluated approximately 72 hours after treatment when there was a consistent difference between the inbreds and the hybrids.

Epinastic response of the hybrids exceeded that of the parents consistently in all three experiments (Table 3). As shown later, the rate of recovery in both hybrid and parents is dependent upon light intensity. An F_2 population was treated in each of these experiments to record the nature of the segregation. Measurements indicated a fair correlation between size of plant and degree of epinasty. (See Appendix).

Because vigor and response to indole acetic acid appeared to be associated, height and epinasty of each individual in the F_2 segregating population were recorded so that the extent of correlation might be determined.

The relationship between the response to indole acetic acid and height was determined and no significant correlation was obtained in Experiment I under low light intensity.

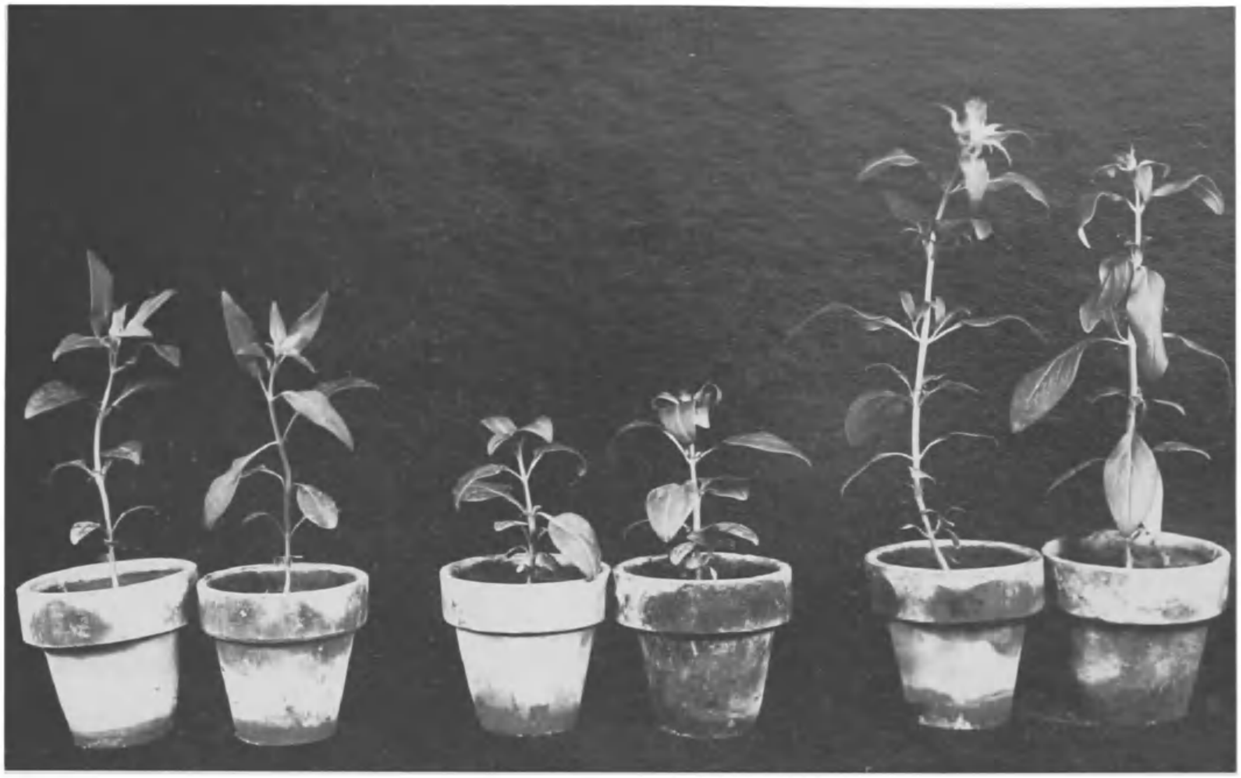

Figure VI

The effect of indole acetic acid on the growth of inbred and hybrid plants of A. majus. The plants on the left in each group were left untreated. The plants on the right were treated with indole acetic acid at 1000 ppm. Group at left P_2, center P_1, right F_1.

TABLE 3

Epinastic response of Parent 1 (P_1), Parent 2 (P_2) and hybrid (F_1) to treatment with indole acetic acid.

	Parent 1 (P_1)	Parent 2 (P_2)	Hybrid (F_1)
Exp I	2	1	3
Exp II	2	1	4
Exp III	1	1	3

Degree of epinasty

0 - no epinasty

1 - very slight epinasty

2 - moderate epinasty

3 - high epinasty

4 - very high epinasty

The epinasty was measured by the amount of curling and distortion of the leaf. Measurements were taken 72 hours after treatment. Response immediately after treatment was very high on both parents and the hybrid.

In Experiment II a correlation of .0738 was found which was significant to the 5% level. This experiment was conducted under moderate light intensities. In Experiment III a correlation of .4206 was found and this was significant to the 1% level. A high degree of correlation was not anticipated since there was an overlapping of the parents and the hybrids in height as shown in Figure I, II and III. The response of the F_2 to indole acetic acid could be correlated with height since most of the plants having a high degree of epinasty were in the larger size classes and the plants showing the least epinasty were the plants in the smaller size classes as shown in the tables on height correlation in the Appendix. This indicates that the plants in the segregating population showing the highest degree of response are of the hybrid genotype. The plants showing the least response and the least height are presumably nearer the parental genotypes.

B. Biological Tests

Since the F_1 consistently showed more epinasty in response to indole acetic acid than either of the parents, it was first thought that this difference was due to a greater production of growth substances in the hybrids, and hence by the addition of an indole acetic acid spray, the hybrids' phytotoxic threshhold would be reached sooner than the inbreds. It was found by chemical analysis that no differences in indole acetic acid could be demonstrated between inbreds and hybrids. Since the hybrids produced more growth it was believed that the indole acetic acid in the hybrids was being utilized for growth more efficiently while in the inbreds its efficiency was reduced perhaps by an inhibitor or an inactivator.

It was found by using the cucumber seedlings test to determine the presence of an inhibitor or an inactivator that the inbreds or P_1 and P_2 had some mechanism of breaking down or inactivating indole acetic acid. (Table 4). The seedlings containing an extract of P_1 and P_2 plus indole acetic acid approached in size the seedlings germinated in distilled water. On the other hand as contrasted to the inhibited growth where seedlings were grown in a solution of indole acetic acid was the cucumber seedlings growing in the extract of the hybrid and indole acetic acid responded in a similar manner to the seedlings germinated in indole acetic acid solution, indicating little breakdown of indole acetic acid where the hybrid extract was used. (Table 4, Figure VII). The seedlings germinated in the dark show a greater response to indole acetic acid than those germinated in the light; a further indication that light is a factor. (Table 4 and 5).

It was found by using the cucumber seedling test that the inbreds in the solution of indole acetic acid approached the distilled water controls. The hybrid in the solution of

TABLE 4

Effect of a simultaneous application of an aqueous extract of either P_1 or P_2 or F_1 and indole acetic acid at 25 ppm. on germination and root growth of cucumber seedlings.

Treatment	I Root growth in mm. Ave. 16 seedlings	II Root growth in mm. Ave. 16 seedlings	Average
Distilled Water	5.8	5.5	5.2
IAA*	0.49	1.0	0.74
Parent 1 + 25 ppm. IAA*	1.95	2.33	2.14
Parent 2 + 25 ppm. IAA*	3.7	4.3	4.00
Hybrid + 25 ppm. IAA*	.47	.56	.51

Seedlings germinated in dark

*indole acetic acid

TABLE 5

Effect of a simultaneous application of an aqueous extract of either P_1 or P_2 or F_1 and indole acetic acid at 25 ppm. on germination and root growth of cucumber seedlings.

Treatment	I Root growth in mm. Ave. 16 Seedlings	II Root growth in mm. Ave. 16 Seedlings	Average
Distilled Water	5.1	4.95	5.02
IAA*	.84	1.09	.96
Parent 1 + 25 ppm. IAA*	1.35	1.51	1.43
Parent 2 + 25 ppm. IAA*	2.99	3.94	3.31
Hybrid + 25 ppm. IAA*	.72	.51	.61

Seedlings germinated in light

*indole acetic acid

indole acetic acid was comparable to the indole acetic acid control. (Figure VII). This indicates that the inbreds have some mechanism for inactivating or breaking down indole acetic acid to a much greater extent than the hybrid.

This will help explain why the inbreds recover from the epinasty when treated with indole acetic acid more rapidly than the hybrids. To determine in what portion of the plant the inhibitor or inactivator was localized or most active, extracts were made of young meristematic tissue as well as extracts of mature tissue. There is more inactivation of indole acetic acid in the meristematic tissue than in the mature tissue. (Table 6).

III. Light Intensity Observations

A. Effect of Light on Heterosis

Since a progressive increase in heterosis was found in successive experiments as the season advanced two curves were plotted to show the concurrent variation in heterosis and solar radiation. (Figure VIII). The solar radiation data were obtained from the United States Department of Agriculture, Hydrologic Research Station, Michigan State College, East Lansing, Michigan and it was found that the solar radiation increased steadily from January through May 1952. (Figure VIII).

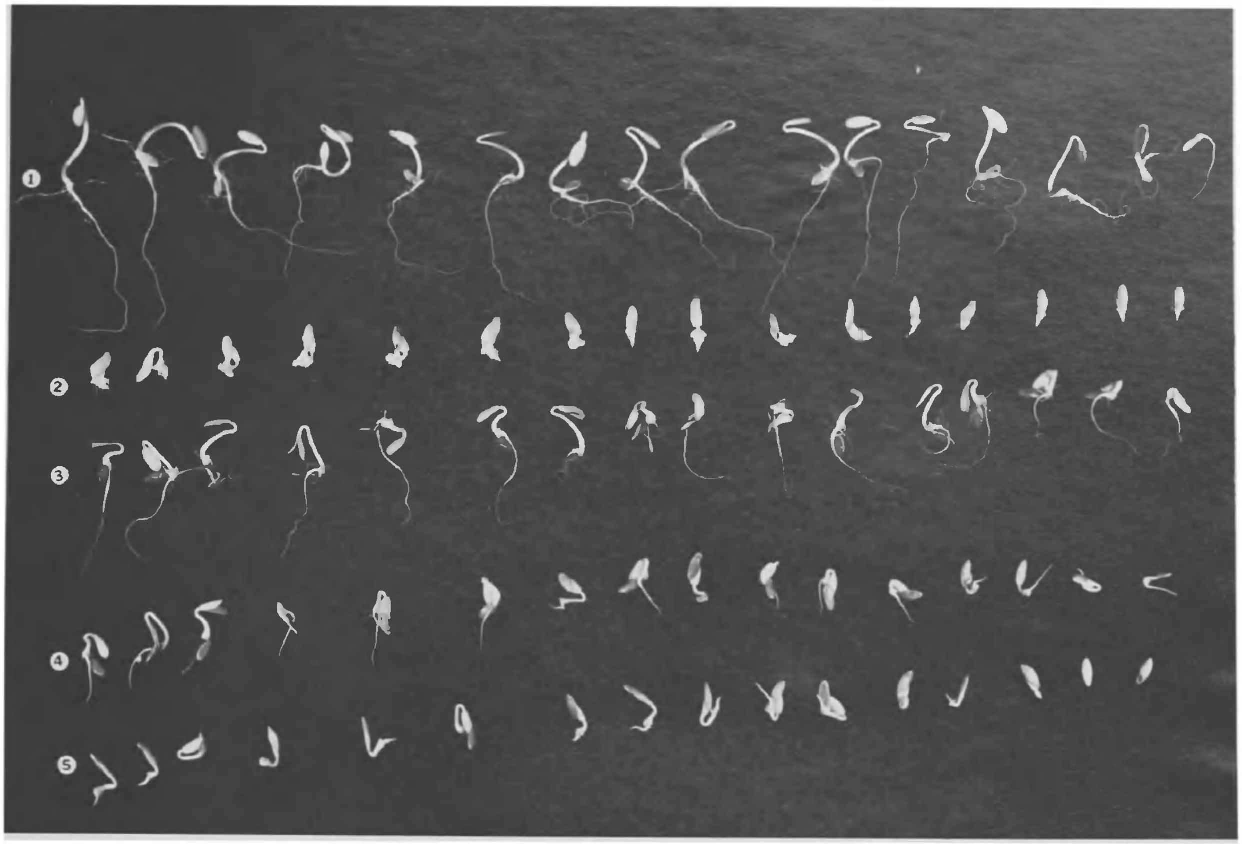

Figure VII

The effect of simultaneous application of an aqueous extract of snapdragon and indole acetic acid at 25 ppm. on the germination and root growth of cucumber seedlings. From top to bottom, 1. distilled water, 2. indole acetic acid at 25 ppm., 3. aqueous extract of P_2 + indole acetic acid at 25 ppm., 4. aqueous extract of P_1 + indole acetic acid at 25 ppm., 5. aqueous extract of F_1 + indole acetic acid at 25 ppm.

TABLE 6

Effect of an aqueous extract of meristematic tissue and of differentiated tissue of P_1, P_2 and F_1 together with indole acetic acid at 25 ppm. on germinations and root growth of cucumber seedlings.

Treatment	I Root growth in mm. Ave. 15 seedlings	II Root growth in mm. Ave. 15 seedlings	III Root growth in mm. Ave. 15 seedlings	Average
Distilled Water	53.6	61.8	64.8	60.1
IAA*	12.6	9.0	13.9	11.8
P_1 meristematic tissue + IAA*	29.4	35.6	34.3	33.1
P_1 differentiated tissue + IAA*	17.1	28.0	32.2	25.7
P_2 meristematic tissue + IAA*	50.4	54.7	56.5	53.8
P_2 differentiated tissue + IAA*	18.1	17.8	30.4	21.8
F_1 meristematic tissue + IAA*	24.0	38.1	42.0	34.6
F_1 differentiated tissue + IAA*	21.0	9.1	55.1	28.3

Seedlings germinated in light

*indole acetic acid

Figure VIII

Increase in solar radiation in gram calories per square centimeters from December 1, 1951 through May 15, 1952 at East Lansing, Michigan.

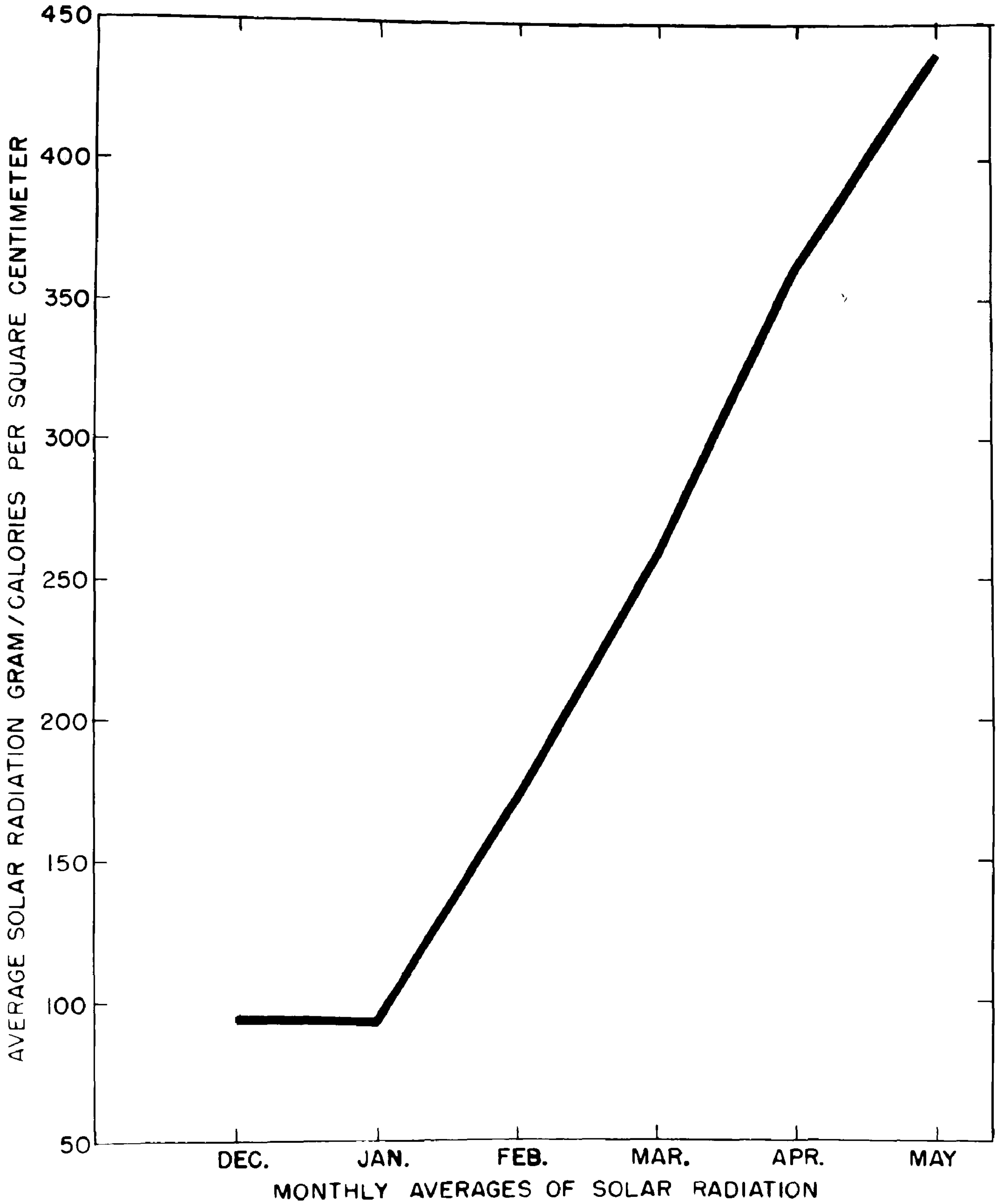
450
400
350
300
250
200
150
100
50
AVERAGE SOLAR RADIATION GRAM/CALORIES PER SQUARE CENTIMETER
DEC.
JAN.
FEB.
MAR.
APR.
MAY
MONTHLY AVERAGES OF SOLAR RADIATION

Figure VIII

In December the total solar radiation was a little less than 100 gram calories per square centimeter and steadily increased to approach a straight line reaching its maximum of approximately 400 gram calories per square centimeter for this experiment in May.

The increase in height of the hybrids over the parents is plotted against solar radiation during Experiments I, II, and III. (Figure IX). The result is very nearly a straight line. As the solar radiation increased the degree of heterosis increased.

Solar radiation was plotted against the dry weight increase of the F_1 in Experiment I, II, and III, and this again gave almost a straight line correlation. (Figure IX). As the solar radiation increased, the difference in dry weight between parents and hybrid increased. Both height and dry weight measurements of heterosis gave almost a perfect correlation with increase in solar radiation as shown by the linearity of the curves.

The fact that the slopes of the two curves are almost identical is evidence of a three way correlation between height, amount of dry weight and solar radiation.

It was found that when the inbreds and hybrid were raised under the same environmental conditions with the exception of light intensity that the inbreds under low light intensity were similar to the hybrid in size. Under high

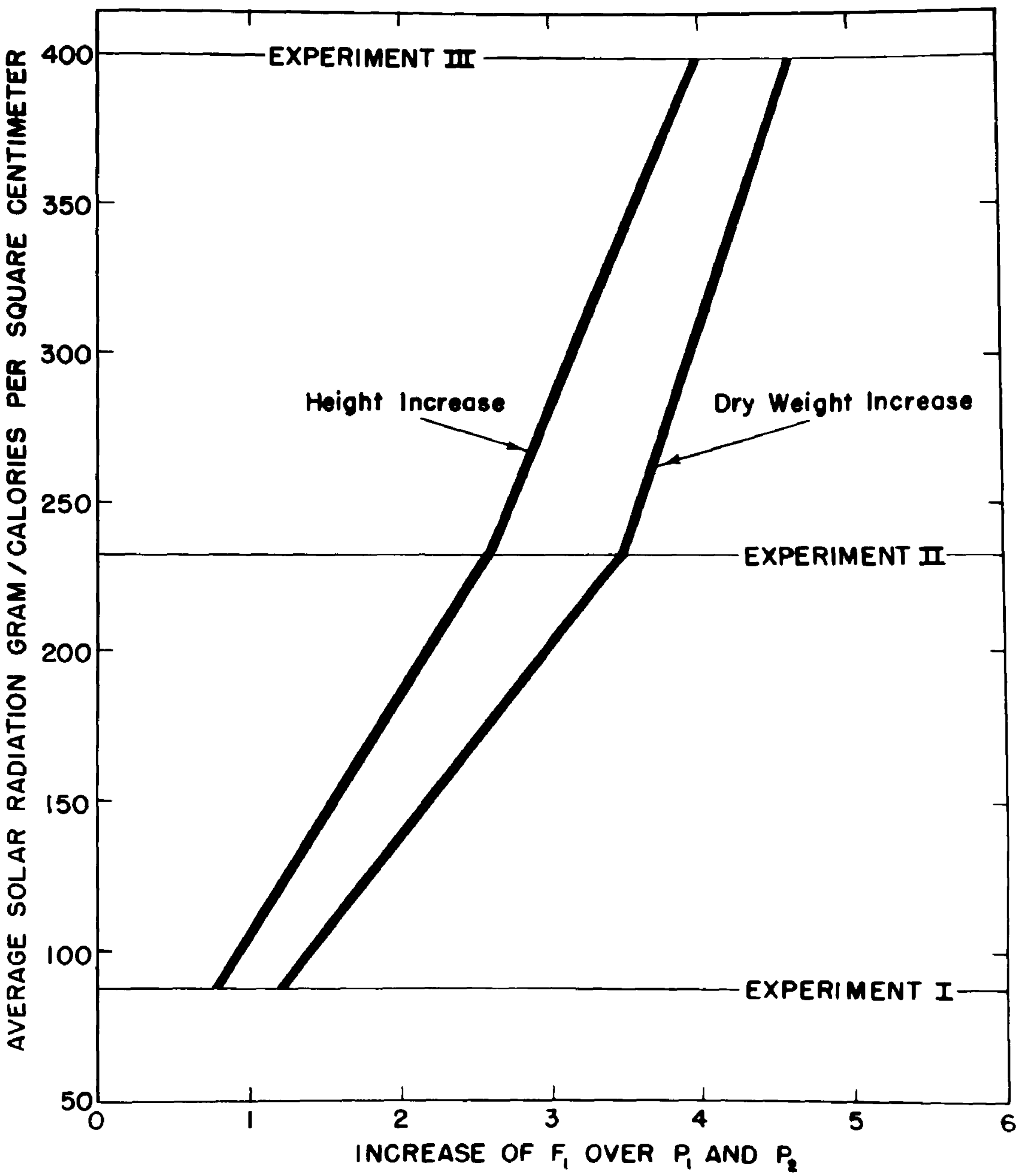

Figure IX

Relationship of the increase in height and dry weight of heterosis to solar radiation.

TABLE 7

Solar radiation in relation to heterosis of *A. majus*.

	Days Grown	Total solar radiation received	Ave. daily solar radiation	Heterosis Ave. dry wt. increase F_1	Ave. ht. increase F_1
Exp I*	62	5512.9	88.9	.115	.73
Exp II**	58	13433.8	231.6	.354	3.65
Exp III***	40	16018.1	400.4	.46	3.95

* Seeds sown December 1, germinated December 6, harvested February 5, 1952.

** Seeds sown February 8, 1952, germinated February 13, 1952, harvested April 6, 1952.

*** Seeds sown April 3, 1952, germinated April 8, 1952, harvested May 12, 1952.

**** Heterosis determined by the increase of the F_1 over the mid-parent mean.

light intensity the inbreds were considerably inferior to the hybrid in size. (Figure X). This again indicates that solar radiation influences heterosis in *A. majus*.

B. Effect of light on the physiological response to indole acetic acid

Treatment of plants in the fall and winter, when natural light is very low, gave response to indole acetic acid in concentrations as low as 5 ppm. As the light intensity increased during the spring the concentrations had to be raised from 100 to 1000 ppm. to obtain the same response. This indicates that this reaction is a photochemical one.

It was found that the parents recovered faster than the hybrid and in each case light received after treatment had a more profound effect on recovery than the light received before treatment. (Figures V and XI). High light after treatment greatly accelerated recovery on all three lines and on plants that had been grown under either high or low light.

Figure XII illustrates the physiological reponse of the inbred and hybrids to two different light intensities. It is clear that plants treated under low light intensity show a greater response than plants treated under a high light intensity.

Figure X

Response of *A. majus* to light intensities. Left to right P_1, P_2, F_1. Top grown at low light. Bottom grown at high light.

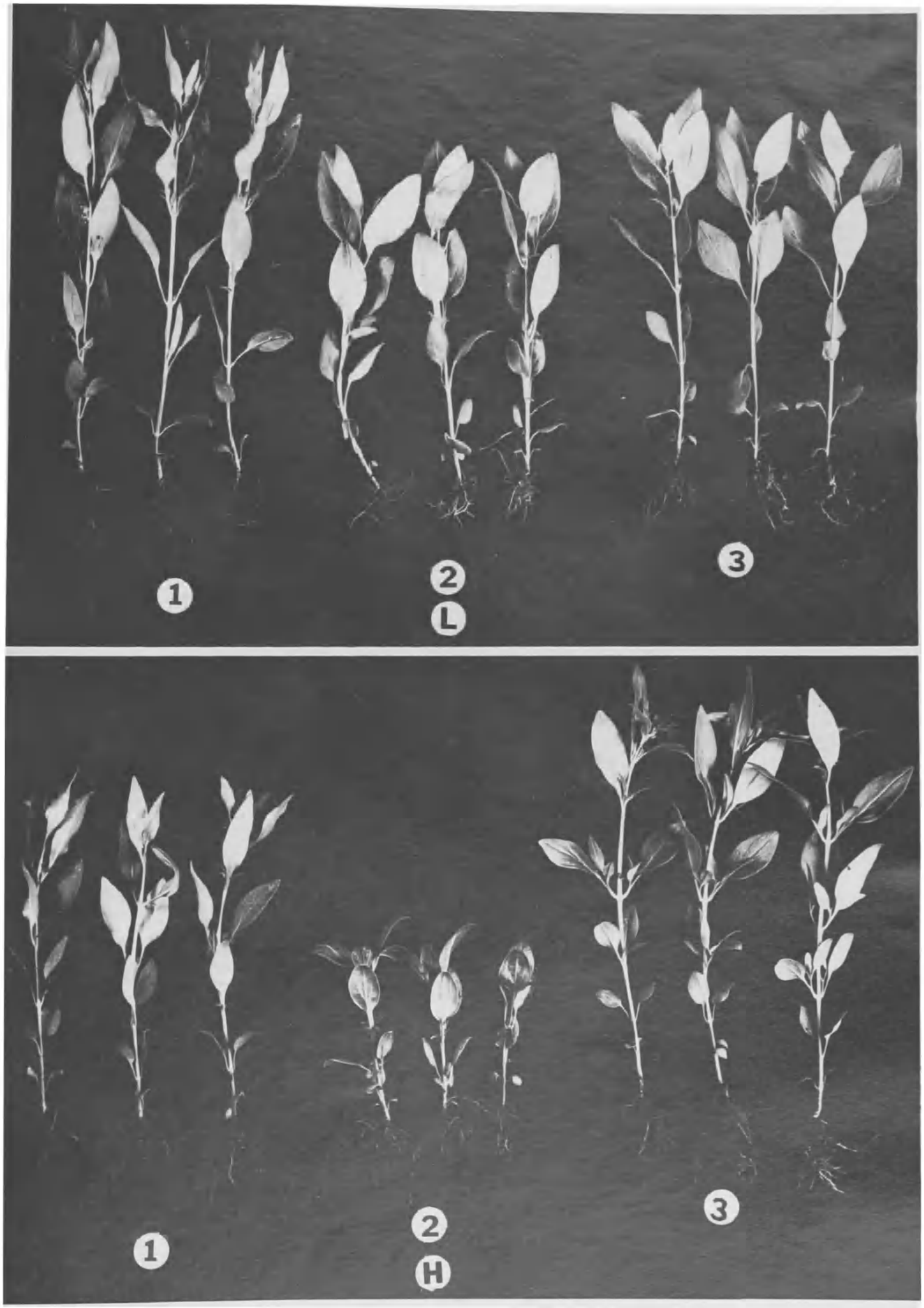

Figure X

Figure XI

Physiological response of *A. majus* to indole acetic acid. Plants on left untreated, plants on right treated. Group one P_2, two P_1, three F_1. Top 72 hours after treatment in high light. Bottom 72 hours after treatment in low light.

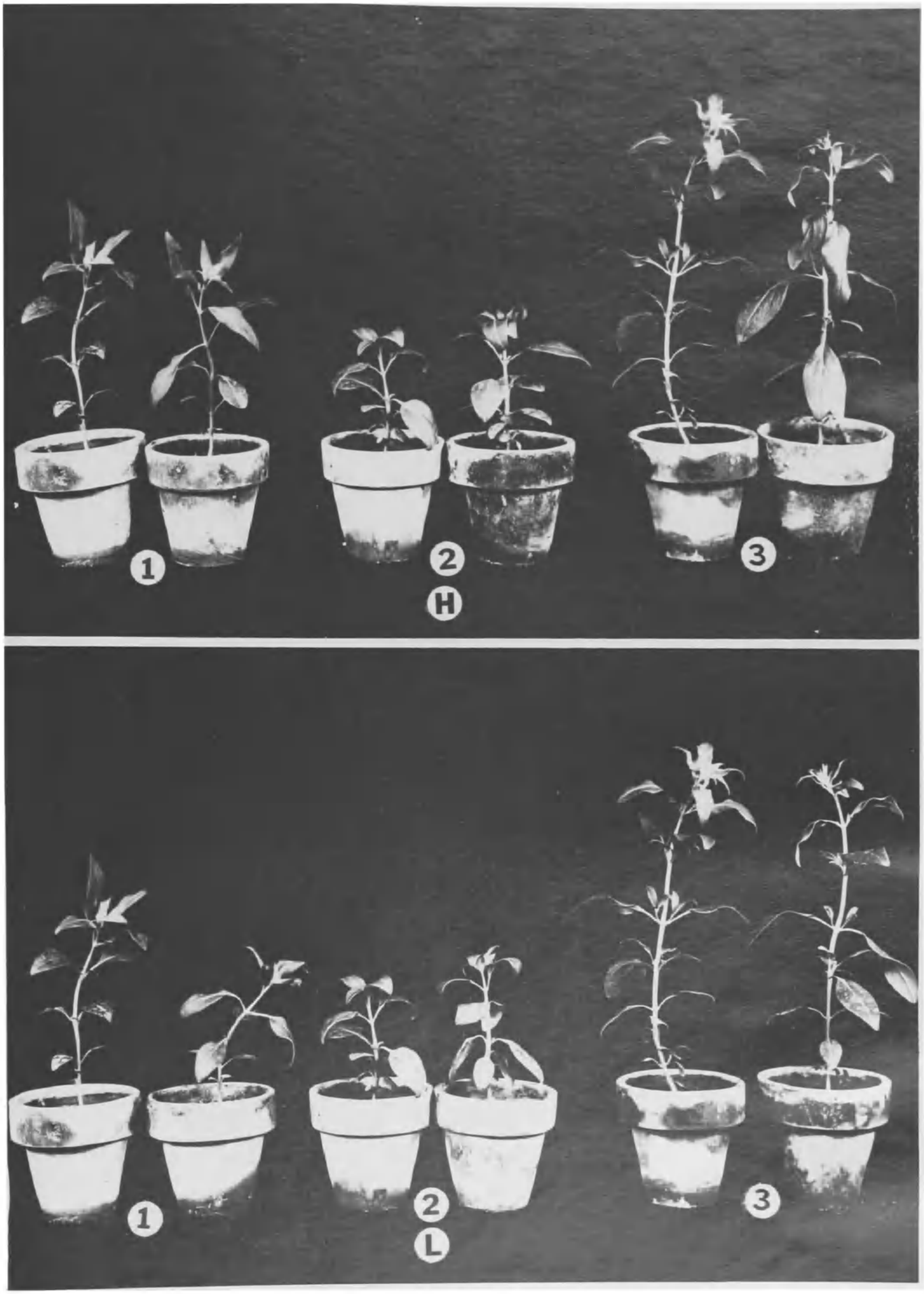

Figure XI

Figure XII

Effect of light intensity on the physiological response of _A_. _majus_ to indole acetic acid. Top P_1, center P_2, bottom F_1. One treated in low light, two treated in high light and three untreated.

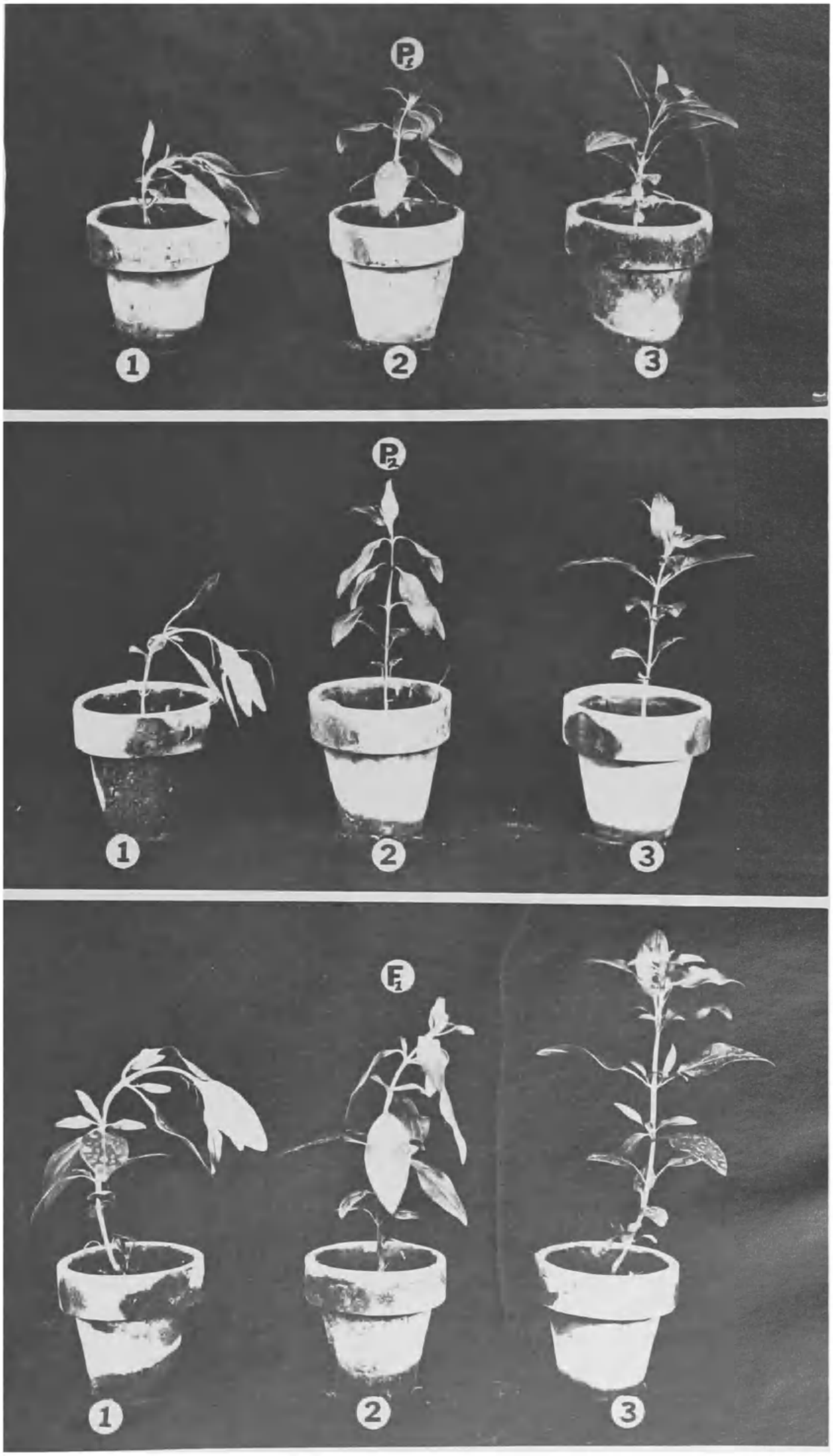

Figure XII

DISCUSSION

During the course of this experiment, some significant facts indicating an interrelationship of indole acetic acid, light intensity and degree of heterosis in A. majus were found.

Heterosis is definitely exhibited in A. majus. In Experiments I, II, and III a positive heterosis was obtained both as to increase in dry weight of the hybrid and as to increase in height of the hybrids. (Tables 1 and 2). The same degree of heterosis was not exhibited at all times. (Figures 1, 2 and 3). In Experiment 1 the degree of heterosis was very slight as compared to Experiments II and III. This variation was consistent as to dry weight increase as well as to increase in height. (Table 1 and 2).

In Experiment I during the winter when light intensity was very low the degree of heterosis was slight. Heterosis increased as the light intensity increased. (Figure IX). This indicates that solar radiation influences heterosis in A. majus. This is substantiated by the linearity of the curve in Figure IX. The expression of heterosis in A. majus can be directly correlated to solar radiation. As the light intensity increases the degree of heterosis increases.

When *A. majus* was raised under low light intensities the differences between the hybrid and the parents were slight. When raised under a high light intensity, this difference was increased as shown in Figure X. This also indicates that the influence of light on heterosis is not a photoperiodic response but a response on the direct increase in light intensity.

When parents and the hybrid were treated with indole acetic acid a differential in epinastic response was obtained. The inbreds recovered from this epinastic response more rapidly than the hybrid. It was found that when the inbreds and hybrids were treated with indole acetic acid under low light intensities that the response was greater than when under high light intensities. When plants were grown under low light the inbreds did not recover from the epinastic response of indole acetic acid as rapidly as when grown under high light. This indicates that the epinastic response of *A. majus* to indole acetic acid can be directly correlated with solar radiation.

By using a biological test, it was found that the inbreds have a greater ability to inactivate or inhibit indole acetic acid than the hybrids. This indicates that the inbreds have a mechanism that inhibits or inactivates indole acetic acid.

Since the inbreds possess a mechanism that inactivates indole acetic acid and also recovers more rapidly when treated with indole acetic acid and the recovery is correlated with solar radiation, that the inactivation or inhibition is also correlated with light.

All evidence seems to point to the fact that the expression of heterosis in A. majus is directly correlated with solar radiation. When A. majus was raised under low light intensities, the difference between the hybrid and parent was very slight. When they were raised under high light intensity, this difference was very significant as shown in Figure X.

Photosynthetic area of a plant may be an important factor in determining the amount of radiant energy that can be used in photosynthesis. The greater ability of hybrids to retain and utilize growth substance under high light conditions permits greater expansion of photosynthetic area. The close relation here demonstrated between growth substance response, solar radiation, and heterosis give abundant evidence for the proposition that heterosis in this hybrid may be primarily due to this mechanism.

As Crabb (10) has shown, the total solar radiation varies from locality to locality and one would not expect the same response from the same crop raised in different localities. It is known that the response of various hybrid snapdragons differs with different localities.

The biological test shows that an inhibitor or an inactivator was present in the inbreds and that this inhibitor in inactivator is not as effective in the hybrids. This could explain why the hybrids are more vigorous than the inbreds under high light intensity. In the inbreds under high light intensities, growth substances are inactivated and in the hybrids the growth substances are not inactivated. As a result of this, the hybrids are able to grow more rapidly than the inbreds. This also explains why under dark cloudy weather characteristic of fall and winter, there is little difference between these inbreds and the hybrids.

The growth substances are not inactivated under low light and the inbreds are permitted to expand more, presenting more leaf area for photosynthetic activity; thus resulting in more efficient growth.

This does not indicate that this is always the case and without a doubt some inbreds of A. majus do not contain this inhibitor or inactivator. They would produce a hybrid that would be excellent under low light conditions. This is the reason why hybrids are limited to areas where they are adapted. This could explain why there are hybrids that respond well in the spring. Indications of this work warrant further investigations along bio-chemical lines aimed at isolating the inhibitor or inactivator. The finding of this inhibitor or inactivator would help give the exact mechanism and the physiological process that is involved. These treatments should be tried with other crops displaying heterosis such as corn, tomatoes, etc. to see if they give the same response.

Genetic analysis of quantitative inheritance has been found difficult in the past. Consideration of the effect of light on the expression of heterosis will reduce unwanted variation between experiments. Since each additional genetic factor increases the minimum population for complete F_2 segregation by four times the isolation of this mechanism from other factors for quantitative inheritance affords considerable simplification of the whole task.

CONCLUSION

1. It was found that *Antirrhinum majus* L. definitely exhibits heterosis.

2. The degree of heterosis exhibited is directly correlated with solar radiation.

3. Treatment with indole acetic acid indicated that hybrids show greater epinastic response than either parent.

4. A biological test showed that the inbreds have a more effective mechanism of inactivating or inhibiting the effects of indole acetic acid.

5. When the inbreds and hybrids are grown under low light intensities, the degree of heterosis is very slight.

6. The epinastic response of *A. majus* to indole acetic acid is directly correlated with light. The inbreds show a higher degree of epinasty under low light than under high light. When the plants are grown under high light, they recover from the epinasty caused by indole acetic acid more rapidly. The hybrids do not recover as rapidly from this response.

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APPENDIX

APPENDIX

I Preliminary Experimentation

To determine the effect of growth substances on two inbreds and their hybrids, seedlings were grown in petri dishes filled with a quartz sand and having three replications per treatment. The quartz sand was treated with the following substances at the rates of 10, 1, 1/10, and 1/100 ppm.:

1. nicotinic acid
2. folic acid
3. riboflavin
4. choline
5. calcium penta thenate
6. pyridoxine hydrochloride
7. thiamine hydrochloride
8. ascorbic acid
9. indole acetic acid
10. 2-3'-5 tri-iota benzoic acid
11. napthalene acetic acid
12. a combination of one through nine
13. a combination of nine through eleven.

This trial was repeated three times.

Growing plants were sprayed with the above compounds. Response to these compounds were observed to determine the compounds effecting heterosis. Seeds were also soaked for a period of twenty-four hours prior to sowing and germinated with and without the above materials. No consistent results were obtained on germination of these substances or by treating the seeds. Only one compound showed a differential correlated with heterosis and this substance was indole acetic acid. Further determinations were made on plants grown in pots with the concentrations being varied from 5 ppm. up to 1000 ppm. After the favorable concentrations were determined plants growing in a greenhouse bench were randomized in groups of forty plants having three replications of each treatment. Since this treatment gave consistent results and there were differences in response between inbreds and hybrids, a greenhouse bench of P_1, P_2, F_1 and F_2 was treated at 100 ppm. of indole acetic acid, Vigor and response to indole acetic acid were recorded.

II Tissue Analysis

To determine the chemical analysis of these plants and to find out if there was any difference between inbreds and hybrids as well as between treated and untreated plants, determinations were made in duplicate on ash, crude fiber, ether extract, protein, nitrogen, calcium, phosphorus, and potassium. Plants used for these determinations were raised in groups of eighty plants and randomized with three replications.

Chemical Analysis of P_1, P_2, and F_1

Sample No.	Ash[1]	Crude Fiber[2]	Ether extract	Protein[3]	N-free extract[2]	Ca	P	K
P_2 check	1418	10.27	2.59	34.81	38.15	2.42	.574	3.44
P_2 IAA*	14.21	10.15	2.65	35.50	37.49	2.38	.566	3.72
P_1 check	15.78	8.40	2.19	37.94	35.69	2.12	.704	3.98
P_1 IAA	15.96	8.58	2.86	37.75	34.85	2.11	.684	4.23
F_1 check	14.45	10.45	2.83	36.06	36.21	2.09	.578	3.75
F_1 IAA	15.03	10.37	3.25	35.94	35.41	2.04	.580	3.86

*Indole acetic acid

[1]Averages of results from duplicate determinations expressed on the oven-dry basis.

[2]The sum of the values for crude fiber and nitrogen-free extract gives an estimation of the total carbohydrate of the tissue.

[3]Kjeldahl nitrogen x 6.25.

III Rivoflavin Data

Since Galston (17) has shown that riboflavin inactivated indole acetic acid in the presence of light and the response of the inbreds indicated that indole acetic acid was inactivated in the presence of light, the tissue was analyzed biologically for riboflavin. Results of these tests are given below:

Riboflavin Analysis of P_1, P_2, and F_1

	Treated*	Untreated*
P_1	13.75	15.26
P_2	13.5	13.46
P_3	13.29	13.32

*values are in milligrams per pound.

IV Cytological Data

To obtain cytological data, meosis and mitosis were studied on P_1, P_2 and F_1 plants treated and untreated with indole acetic acid. Meosis and mitosis were apparently normal. All are diploid with eight pairs of chromosomes.

V Respiration Data

Respiration studies were made on germinated seedlings by using the Warburg method (47, 48) and growing plants were measured for respiration by the Ulrich (45) method and no differences were obtained between treated or untreated or between inbreds or hybrids.

VI Fresh and Dry Weights of Treated and Untreated Plants

To determine differences in fresh and dry weights of treated and untreated plants, lots of 350 plants each of P_1, P_2, and F_1 were treated, and average weights were obtained. Results are given in the table below:

Dry Weight Harvested March 19, 1952

Exp I — Average of 350 plants

	Average fresh wt. per plant	Average dry wt. per plant
P_2 check	3.6	.371
P_2 treated IAA*	3.48	.357
P_1 check	2.15	.195
P_1 treated IAA*	2.34	.216
F_1 check	3.93	.401
F_1 treated IAA*	4.47	.394

*indole acetic acid

Dry Weight Harvested April 14, 1952

Exp II Average 350 plants

	Average fresh wt. per plant	Average dry wt. per plant
P_2 check	2.48	.206
P_2 treated IAA*	2.38	.225
P_1 check	2.85	.231
P_1 treated IIA*	3.07	.252
F_1 check	6.42	.57
F_1 treated IAA*	5.64	.539

Dry Weight Harvested May 16, 1952

Exp III Average 350 plants

	Average fresh wt. per plant	Average dry wt. per plant
P_2 check	2.44	.16
P_2 treated IAA*	2.52	.2
P_1 check	1.6	.12
P_1 treated IAA*	1.88	.16
F_1 check	6.8	.6
F_1 treated IAA*	5.32	.54

*indole acetic acid

No significant differences were obtained in the measurements of these fresh and dry weights between treated and untreated plants.

VII Solar Radiation

Raw data for the solar radiation from December 1951 through May 1952 which was used in making Graph IV is presented below:

Solar Radiation gram calories per sq. cm.

December 1, 1951 - May 15, 1952

Weekly Ave.	December 1951	January	February	March	April	May
Total 1-5	611.30	487.5	328.40	905.60	736.70	2770.0
Ave.	102.26	97.5	65.68	181.12	147.34	554.0
Total 6-10	413.5	344.6	623.60	1349.0	1748.90	1904.1
Ave.	82.7	68.92	124.72	269.8	349.78	380.8
Total 11-15	424.5	395.70	1070.60	1537.80	1142.30	1916.4
Ave.	84.9	79.14	214.12	307.56	228.46	383.3
Total 16-20	529.60	407.70	1024.0	1015.90	2546.10	
Ave.	105.92	81.54	204.8	203.18	509.25	
Total 21-25	401.80	507.40	1222.70	1412.40	1829.40	
Ave.	80.36	101.48	244.54	282.48	365.88	
Total 26-30	539.40	732.5	763.30	1808.10	288.01	
Ave.	89.9	122.08	190.83	301.35	57.60	
Monthly Total	2920.4	2875.4	5032.6	8028.8	10883.5	
Monthly Average	94.2	92.8	173.5	259.0	362.8	

VIII Data for Correlation of Vigor and Response to Growth Substances.

The following tables are the height and response to indole acetic acid data that was used in obtaining the correlation.

Experiment I

Correlation of height versus response of segregating population (F_2) to indole acetic acid.

Response to indole acetic acid	Vigor								
	4	5	6	7	8	9	10	11	12
0	2	3	4	6	6	4	6	2	
1	1	9	8	7	6	13	14		1
2	3	2	7	5	13	9	5	3	2
3	1	1	4	8	3	2	3		
4		1	1						
5									

Experiment II

Correlation of height versus response of segregating population (F_2) to indole acetic acid.

Response to indole acetic acid	Vigor											
	3	4	5	6	7	8	9	10	11	12	13	14
0			1	1	7	10	11	5	10	6		
1		2	2	3	10	17	26	32	28	18	6	1
2			2	5	11	23	50	57	50	37	13	3
3		1	2	5	14	27	46	49	43	35	9	3
4	1	1	1		5	7	15	16	14	10	3	1
5							1	1	1	2		

Correlation of height versus response of segregating population (F_2) to indole acetic acid.

	Vigor										
Response to indole acetic acid	3	4	5	6	7	8	9	10	11	12	13
0	2	4	7	7	7	3					
1	8	27	40	47	42	14	7	2	1		
2	4	25	43	74	58	34	17	8			
3		9	35	89	106	75	46	17	6		
4	1	3	10	34	48	52	42	25	5	3	1
5			2	2	9	10	5	2	4		1