EFFECT OF GROWTH RETARDANTS ON PHASEOLUS VULGARIS

SOME ASPECTS OF THE EFFECT OF GROWTH RETARDANTS ON PHASEOLUS VULGARIS

By

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TITLE: Some Aspects of the Effect of Growth Retardants on *Phaseolus vulgaris*

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SCOPE AND CONTENTS: A quantitative study is made of the relative sensitivity of *Phaseolus vulgaris* var. Pinto to several different growth retardants, and to treatment of retarded plants with GA_3. The growth of progeny from retarded plants is examined and the cause of the transfer phenomenon is determined to be transfer of the growth retardants themselves to the progeny *via* the seeds, in some cases through to a third generation.

PREFACE

This thesis describes work carried out in the Research Unit in Biochemistry, Biophysics, and Molecular Biology from May, 1964 to December, 1965.

I am grateful to several people who have helped to bring this work to completion--to Dr. S. F. H. Threlkeld, head of the Research Unit, for urging me to begin, to J. A. for his constant encouragement to continue, and to my mother for her hours of willing work in typing the manuscript. Special thanks are due my supervisor, Dr. J. A. D. Zeevaart for his continuing interest and invaluable advice concerning the work itself and the preparation of the manuscript.

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CHAPTER I

Introduction

Growth retardants have been defined as "all chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically, without formative effects." (Cathey, 1964). Preston (1961) and Bukovac (1964) described the effects of two types of growth retardants on the gross morphology of Phaseolus vulgaris. Typical dwarfed plants had dark-green foliage, short, thick internodes, and restricted petiole expansion. These characteristics are typical of all plants dwarfed by the growth retardants.

The compounds so far discovered which induce these responses in plants are of several types. Certain substituted cholines (Tolbert, 1960), quaternary ammonium carbamates (Wirwille and Mitchell, 1950; Preston, 1961; Cathey, 1965), phosfoniums (Preston and Link, 1958) and 1,1 dimethylhydrazide derivatives of succinic and maleamic acid (Riddell et al, 1962) have been shown to have growth-retarding activity.

The spectrum of sensitivity of plants to growth retardants, as measured by decrease in stem elongation, varies greatly. A species which is sensitive to one chemical will not necessarily be affected by others, even though they may be closely related analogues. (Zeevaart and Osborne, 1965). Cathey (1965) tested fourteen quaternary ammonium

carbamates closely related to Amo-1618[1] on 28 species of plant. Within the same genus, all cultivars and species tested exhibited a similar spectrum of selectivity to the chemicals, but at higher organizational levels no relationship between phylogenetic proximity and sensitivity could be observed. It was postulated that these differences were due to species variation in the enzymes of the system or systems affected by the growth retardants.

At the cellular level, the mode of action of growth-retarding chemicals has been studied by Sachs et al (1960),and Sachs and Kofranek (1963). Amo-1618, CCC, and Phosfon-D inhibited subapical cell expansion and division in intact plants of Chrysanthemum morifolium. External application of GA completely reversed the inhibition.

This reversal of their effect on stem growth by GA is a universal characteristic of the growth retardants. In Phaseolus vulgaris the

[1]The following abbreviations will be used:

Amo-1618: 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride.

Carvadan: 3-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride.

Phosfon-D: 2,4-dichlorobenzyl tributyl phosfonium chloride.

CCC (Cycocel): (2-chloroethyl) trimethylammonium chloride.

B-995: N-dimethylaminosuccinamic acid.

CO-11: N-dimethylamino maleamic acid.

GA: Gibberellin.

IAA: Indoleacetic acid.

reversal of growth-retardant induced inhibition by GA has been observed for the compounds Amo-1618 (Downs and Cathey, 1960), CCC and Phosfon-D (Lockhart, 1962) and CO-11 (Bukovac, 1964).

Since in Fusarium the retardants CCC and Amo-1618 block biosynthesis of GA without inhibiting growth of the mycelium (Kende et al, 1963; Ninnemann et al, 1964), it has been suggested that growth retardants act in higher plants by blocking the biosynthesis of endogenous GA, and the external application of GA is thus able to overcome the deficiency in native GA's.

In support of this hypothesis, Baldev et al (1965) reported that gibberellin content in excised pea pods cultured in the presence of Amo-1618 was significantly reduced. This was the case even at the lowest concentration used, where growth of the seeds was not affected, thus ruling out GA reduction as a secondary effect of growth inhibition. In wheat embryos, CCC reduced significantly the level of endogenous GA (Michniewicz, 1965), and in the case of Pharbitis nil, GA-activity in seeds from CCC-treated plants was reduced up to 80% as compared to that in untreated control plants (Zeevaart, 1966). Köhler (1965) found that light-grown seedlings of Pisum sativum without CCC contain more than 10X as much GA-activity as plants grown with CCC, and showed that this loss of GA is the cause and not the effect of the growth depression by CCC. These results all indicate that growth retardants do act in higher plants by blocking GA biosynthesis.

Phaseolus vulgaris is a species about which a considerable amount of information is available. As has been previously mentioned, it shows a wide range of sensitivities to growth retardants of all types

--quaternary ammonium carbamates (Wirwille and Mitchell, 1950; Marth et al, 1953), phosfoniums and substituted cholines (Lockhart, 1962); and the 1,1 dimethylhydrazide of succinic maleic acid (Bukovac, 1964). In all these cases the effect of the growth retardants on stem elongation is completely reversed by external application of GA.

GA alone applied to normal plants causes an increase in height. However in the presence of the growth retardant Amo-1618, no increase in growth over the normal height could be induced with GA (Downs and Cathey, 1960).

An interesting effect of the growth retardant Amo-1618 and some of its analogues on P.vulgaris was transfer of the "dwarfing response" to succeeding generations (Marth et al, 1953; Preston, 1961). Dual treatments of Amo-1618 and GA applied in lanolin pastes to the first internode resulted in only the Amo-1618 response being manifested in the progeny (Preston, 1961). The application of Amo-1618 alone to the flower peduncle also resulted in dwarfing of the progeny. Thus, the parent plants need not be dwarfed to give rise to dwarfed progeny. The mechanism of transfer of the response, and the stage of development at which it is transferred, are not known.

The naturally occurring gibberellins may be synthesized in the stem tip, as suggested by Lockhart from his experiments on peas (1957), or in the root, as suggested by Phillips and Jones from work with Helianthus annuus (1964), or perhaps in some other part of the plant. In any case they are known to be translocated readily in the plant, moving both in xylem and phloem (Zweig et al, 1961). They have been identified by Jones (1964) for Phaseolus multiflorus using thin-layer

chromatography of seed extracts. GA_1, GA_3, GA_5, GA_6, and GA_8 appeared at maxima of 3.5, 0.2, 0.6, 0.5 and 9.0 μg/seed, respectively.

Two gibberellins have been identified in extracts of Phaseolus vulgaris. West and Phinney (1959) detected two substances, bean factor I and bean factor II, in immature seeds of P.vulgaris which were later shown to be identical with GA_1 and GA_5, respectively (Phinney and West, 1960).

For P.vulgaris, total GA content of the seed was determined by Corcoran and Phinney (1962) using a dwarf corn d-1 bio-assay (where GA_3 is most active and GA_5 relatively inactive) and was found to reach a peak of 1.0 μg GA_3-equivalents/1000 seeds. Skene and Carr (1961) used a dwarf pea bio-assay and detected 17 μg GA_3-equivalents/1000 seeds in each of two active zones.

From the work thus far done on GA content, it appears that P.multiflorus has a higher level of endogenous GA than P.vulgaris, which would make it a better system to use for study of GA-growth retardant effects. However, the phenomenon of transfer of dwarfing to the progeny did not occur in this species as it did with P.vulgaris (Preston, 1961). In those species which did show transfer of the dwarfing response, flower opening was delayed several days, while in P.multiflorus, flowering occurred two weeks earlier than in control plants. This species therefore was not a promising one for study of the transfer phenomenon. Phaseolus vulgaris, on the other hand, has several properties which make it a potentially excellent system for study of the mode of action of growth retardants, and of GA in reversing the effect:

1. It is sensitive to treatment with growth-retarding chemicals of all types.

2. The effect can be reversed by external application of GA.

3. The dwarfing response induced by Amo-1618 analogues can be transferred to progeny via the seeds.

4. The seeds contain measurable amounts of GA.

Scope of this Investigation

This investigation consisted of

I. a quantitative study

(1) of the relative sensitivity of Phaseolus vulgaris var. Pinto to several growth retardants.

(2) of the response of retarded plants to GA_3.

(3) of the effect of the time of GA_3 treatment on the response to GA_3.

(4) of the growth of the progeny from dwarfed parent plants.

II. a study of the phenomenon of transfer of the dwarfing response

(1) by determination of the nature of the GA's present in seeds from treated and untreated plants.

(2) by experiments to detect the presence of growth retardants in seeds from treated plants.

CHAPTER II

Materials and Methods

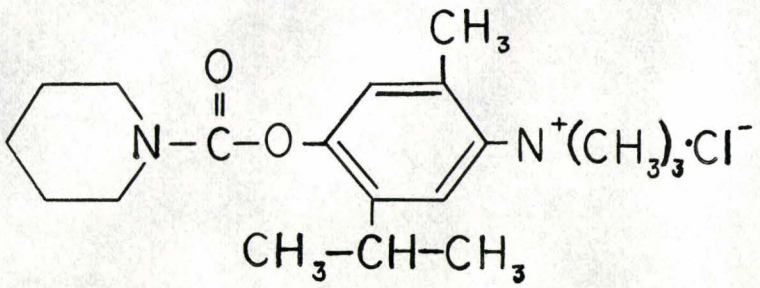
1. Chemicals

The compounds used in this study were Amo-1618 and its position isomer called Carvadan (Halevy, 1962), CCC, B-995, and Phosfon-D. The chemical names and structures of these compounds are given in figure 1. They are representative of the four main types of growth-retarding chemicals (chapter I).

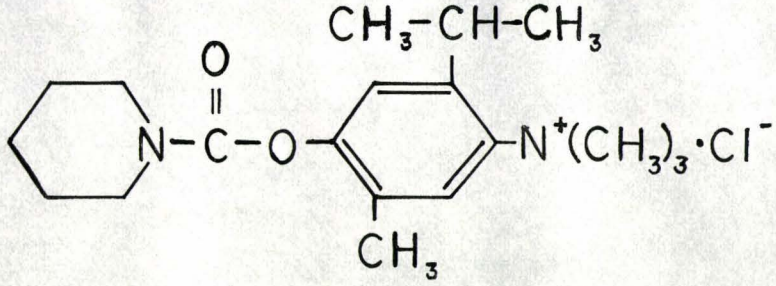
Amo-1618 was obtained from Rainbow Color and Chemicals Co., Sepulveda, California. Carvadan was kindly provided by Dr. H. M. Cathey, Plant Industry Station, Beltsville, Md. CCC was obtained as a gift in an 11.8% solution under the trade name Cycocel, from American Cyanamid Company, Princeton, N.J.; Phosfon-D as a 10% solution from Virginia-Carolina Chemical Corporation, Richmond, Va.; and B-995 was kindly provided by Dr. J. A. Riddell, United States Rubber Co., Naugatuck, Connecticut. Samples of pure gibberellins were supplied by Dr. D. F. Jones, Imperial Chemical Industries, Welwyn, Herts., U.K.

2. Growth of the Plants

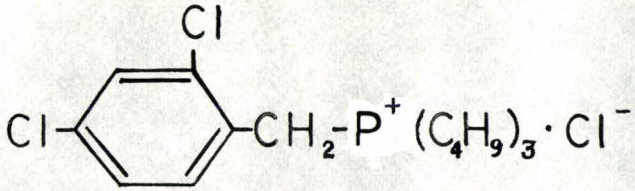
Plants of Phaseolus vulgaris, var. Pinto (Atlee Burpee Co., Philadelphia) were grown in 320 ml or 950 ml plastic containers filled with a mixture of vermiculite (medium size) and coarse sand (size 3). They were watered daily at first, and twice daily as they grew larger, with half-strength Hoagland solution containing 10 ppm Fe as Na-Fe sequestrene (Appendix p.60).



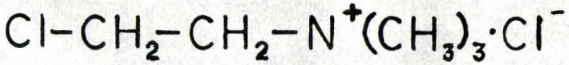
Amo-1618



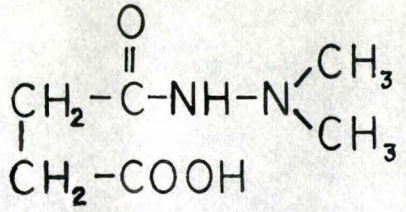
Carvadan



Phosfon-D



CCC



B-995

Figure 1. Chemical structures of five growth retardants.

All experiments with intact plants were conducted in growth rooms. Light was provided by Sylvania cool white VHO fluorescent tubes, supplemented by three 150 W incandescent bulbs every 16 tubes. The two types of lamps were controlled by separate time switches.

Plants were raised from seed at 25°C under continuous fluorescent light until the second internodes (between primary leaves and first trifoliate leaf) started to elongate. Uniform plants with second internodes 2 to 3 mm long were selected and transferred to a growth room kept at a constant temperature of 25°C and a photoperiod of 10 hours consisting of 8 hours fluorescent light (900 ft-c at plant level), followed by 2 hours incandescent light (approximately 150 ft-c). This light regime was chosen to obtain maximal stem elongation with the light sources available (Downs et al, 1957; Lockhart, 1964). Figure 2 shows the effect of three different light regimes on stem elongation. The short day of 8 hours fluorescent + 2 hours incandescent obviously caused greatest elongation, thus showing the greatest contrast between treated and control plants.

3. Application of Chemicals

(i) Growth Retardants

Immediately after selection and transfer, the plants were treated by a single application of 10 ml of the test solution, or of water in the case of controls, via the roots. During the next few days, nutrient solution was given sparsely to all plants so that the containers were never drained. The length of the second internode and total stem length above the primary leaves were measured at regular intervals until termination of the experiment. In all experiments there were 8 plants per treatment unless stated otherwise.

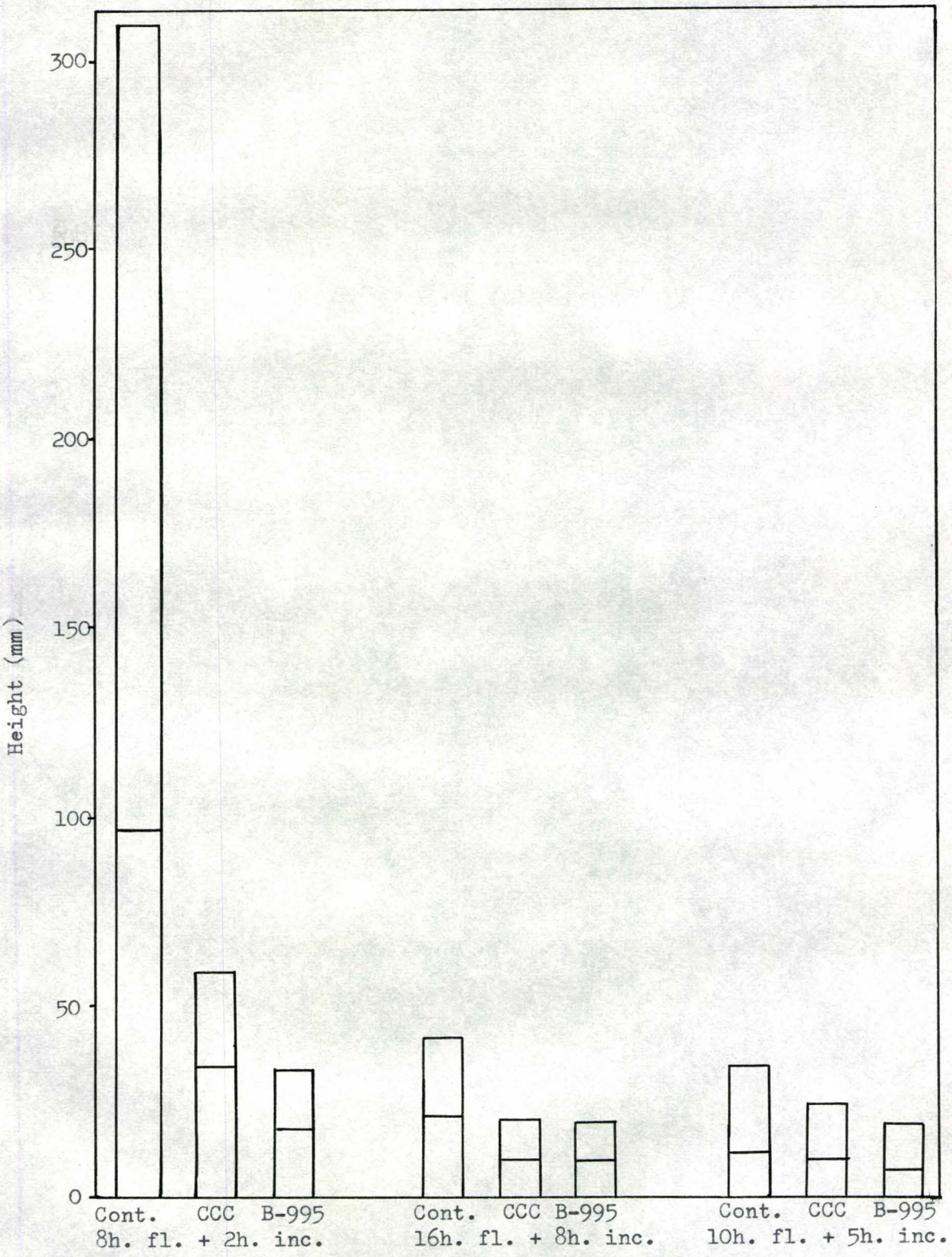


Figure 2. Effect of light conditions on growth of Phaseolus vulgaris. Total stem length above primary leaves measured after 8 days.

(ii) Gibberellins

GA_3 was applied dissolved in an aqueous solution of 0.05% Tween 20. With the aid of a microsyringe, 10 µl was applied in tiny droplets. When gibberellin treatment was on the day of transfer and treatment with growth retardant, the application was made to the primary leaves, which were approximately half expanded at this stage. When GA treatments took place later than the day of treatment with the growth retardant, the application was made to the trifoliate leaf which was approximately half expanded at that time.

4. Testing the effect of growth retardants on progeny

For these experiments, plants were grown under the conditions described in the larger 950 ml containers, since they were to be grown to maturity. On the first day that flower buds were visible (approximately 1 month after planting), the growth retardants were applied via the roots, 10 mls per plant. This treatment was repeated at weekly intervals. Flowers were tagged on the day of anthesis, 6 per plant (2 per node on each of three successive nodes). Later flowers were removed.

(i) Effect on seed growth

At regular intervals after the day of anthesis, fruits were collected and the seeds removed and counted. They were weighed, and frozen in liquid nitrogen. After lyophilization, dry weight was determined. Lyophilized seeds were stored in stoppered vials at -23°C until extraction. Mature seeds were also collected and stored at room temperature (20°C) until planting. This progeny was designated P_1.

(ii) Effect on growth of progeny

To test for dwarfing in the progeny, 10 seeds from control

plants and from each of the treatments were weighed and sown in 950 ml containers according to the usual procedure. Length of the second internode and total length were measured at regular intervals after the plants were transferred to the 8 hr + 2 hr light regime. Mature seeds (P_2) were collected again from these plants, and the procedure was repeated with these to give P_3. Thus, three generations from the treated plants were studied.

To obtain good seed set from P_1, it was necessary in the case of Carvadan progeny to treat the plants with 0.1 µg GA_3, as described above. This would have no effect on growth of the progeny (Preston, 1961).

5. Extraction of seeds for GA-like substances

Seeds of P_1, which had been collected at regular intervals after the day of anthesis, were ground up in cold methanol and extracted three times with methanol at 3°C and once at room temperature. The methanol extracts were combined and the volume reduced to approximately 1 ml under vacuum. Ascending thin-layer chromatography (TLC) of the extracts was carried out on 400µ thick plates of Silica gel H. The plates were developed in di-isopropyl ether/acetic acid (95:5) for 15 cm. (MacMillan and Suter, 1963), divided into 1.5 cm horizontal bands, and scraped into 12 ml centrifuge tubes. Each of the ten fractions was eluted 2X with 3 ml water-saturated ethyl acetate (Kende and Lang, 1964). The eluates were removed by low-speed centrifugation. Supernatants were combined in disposable 50 ml plastic beakers and dried under a fan. The dried samples were then stored at -23°C until being tested for GA-like activity.

To determine the kinds and amounts of gibberellin normally present in seeds of pinto beans, an extraction was carried out on a large quantity of immature seeds (300) from plants grown in the field. The seeds were first weighed, lyophilized, and weighed again. A partial purification was then carried out following the procedure of Kende and Lang (1964) as follows: The dried seeds were ground up in methanol using a mortar and pestle. The resultant slurry was extracted with methanol three times at 3°C and once at room temperature on a reciprocal shaker. The extracts were combined and dried down under reduced pressure with the aid of a rotary evaporator. The residue was taken up in 0.1M phosphate buffer (pH 8.2) and petroleum ether (B.R.38°-53°C) and partitioned with petroleum ether until the organic phase was colourless. The buffer phase was then extracted twice with ethyl acetate. The pH was adjusted to 2.5 with 6N HCl and an acidic fraction was obtained by partitioning three times with equal volumes of ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This acidic fraction was taken up in ethyl acetate and pipetted onto the column which had been prepared as follows: 18 grams of washed Celite were packed in a glass column (height 300 mm, I.D. 17 mm) to a height of 20 cm, using a stainless steel tamper. 75 mls of 0.5M phosphate buffer at pH 6.4 was shaken with 150 mls ethyl acetate and passed through the column using suction. When the column was almost dry, the acidic fraction in ethyl acetate was pipetted onto the column. The column was developed with 500 mls of dry ethyl acetate and washed with 30 mls methanol. Ten 50-ml fractions were collected, evaporated under reduced pressure, transferred to plastic

beakers, and dried under the fan. They were then stored at -25°C until being tested for GA-like activity.

TLC of these extracts was carried out on plates of Silica gel H, 250μ thick. The dried extracts were redissolved in 1 ml ethyl acetate, and 10 μl of each were applied to the plate. Standard solutions of GA_1, GA_3, GA_4, GA_5, and GA_9 were co-chromatographed. The solvents used were di-isopropyl ether/acetic acid (95:5)(MacMillan and Suter, 1963), iso-propanol/water (4:1)(Elson et al, 1964), and chloroform/ethyl acetate/ acetic acid (60:40:5)(Sembdner et al, 1962). When dry, the plates were sprayed with ethanolic sulphuric acid (95:5) and heated at 120°C for ten minutes. R_f values and fluorescence of the spots under U.V. light were observed and compared with the standards and with results reported in the literature.

6. Bio-assays of extracts for GA-like substances

(i) Dwarf pea bio-assay (Köhler and Lang, 1963).

Seeds of dwarf peas (Pisum sativum var. Progress no.9) were obtained from Asgrow Seed Co., New Haven, Conn. They were soaked overnight in water containing a small amount of the fungicide Captan, and planted in vermiculite the next day. After 3 days, seedlings of uniform height (16-20 mm) were selected and transferred to half-strength Hoagland's solution. The samples to be tested, dissolved in an aqueous 0.05% Tween 20 solution,and the standard solutions were applied in 5 μl drops to the plumular hook with a microsyringe, 8 plants per treatment. In the case of extracts from immature seeds, the drops were applied twice to give a greater dose, since the samples were already dissolved in a minimal amount of an aqueous 0.05% Tween 20 solution. After treatment,

the plants were transferred to continuous weak red light for five days. At the end of this time, the length of the stem from top of the radicle to apex was measured.

(ii) Dwarf corn bio-assay (Phinney, 1961).

Seeds of dwarf corn d-1 and d-5 were soaked for 24 hours and planted in vermiculite. After 7 days, dwarf plants were selected as the funnel of the first leaf opened, and transferred to ½ strength Hoagland's solution, 0.1 ml of the test solution was applied in the funnel of the first leaf. The plants were grown in a growth chamber at 27°C with 8 hours light per day for 7 days, after which the lengths of the first and second leaf sheaths were measured.

7. Extraction of growth retardants from seeds

Growth retardants were extracted from the seeds with methanol. The methanol extraction was repeated several times, and the resultant extract was evaporated under reduced pressure and redissolved in 90% methanol.

TLC was carried out on cellulose plates (250μ) in butanol/acetic acid/water (BAW) (4:1:1.8) for 10 cm. The plates were then sprayed with Dragendorff's reagent (Appendix p.61) and the R_f and intensity of the resultant spots compared with those of standard series applied to the same plate.

CHAPTER III

The Effect of Growth Retardants on Growth in Intact Plants

1. Relative sensitivity to growth retardants

Phaseolus vulgaris was tested for its sensitivity to four different growth retardants. The results of these experiments are shown in figure 3. The order of effectiveness is Amo-1618 > Phosfon-D > B-995 > CCC. The relative amounts of each required to cause 70% inhibition, with Amo-1618 represented by 1, are 1:5:300:1500 respectively. Thus 1500X more CCC than Amo-1618 is required to cause 70% inhibition. All four curves show a similar trend, and a saturation level is reached beyond which higher doses give no greater response.

The appearance of plants treated with the growth retardants was similar to that described by others (Preston, 1961; Bukovac, 1964). All retarded plants had short thick internodes and dark green leaves of approximately the same size as those of untreated control plants.

2. Reversal of inhibition with GA

(i) Concentration of GA. When GA_3 was applied simultaneously with the growth retardants, reversal occurred as shown in figure 4. The concentrations of growth retardants used were chosen to give less than the maximum inhibition in each case. They were:

Amo-1618	3×10^{-5} M
Phosfon-D	8×10^{-5} M
B-995	5×10^{-3} M
CCC	5×10^{-2} M

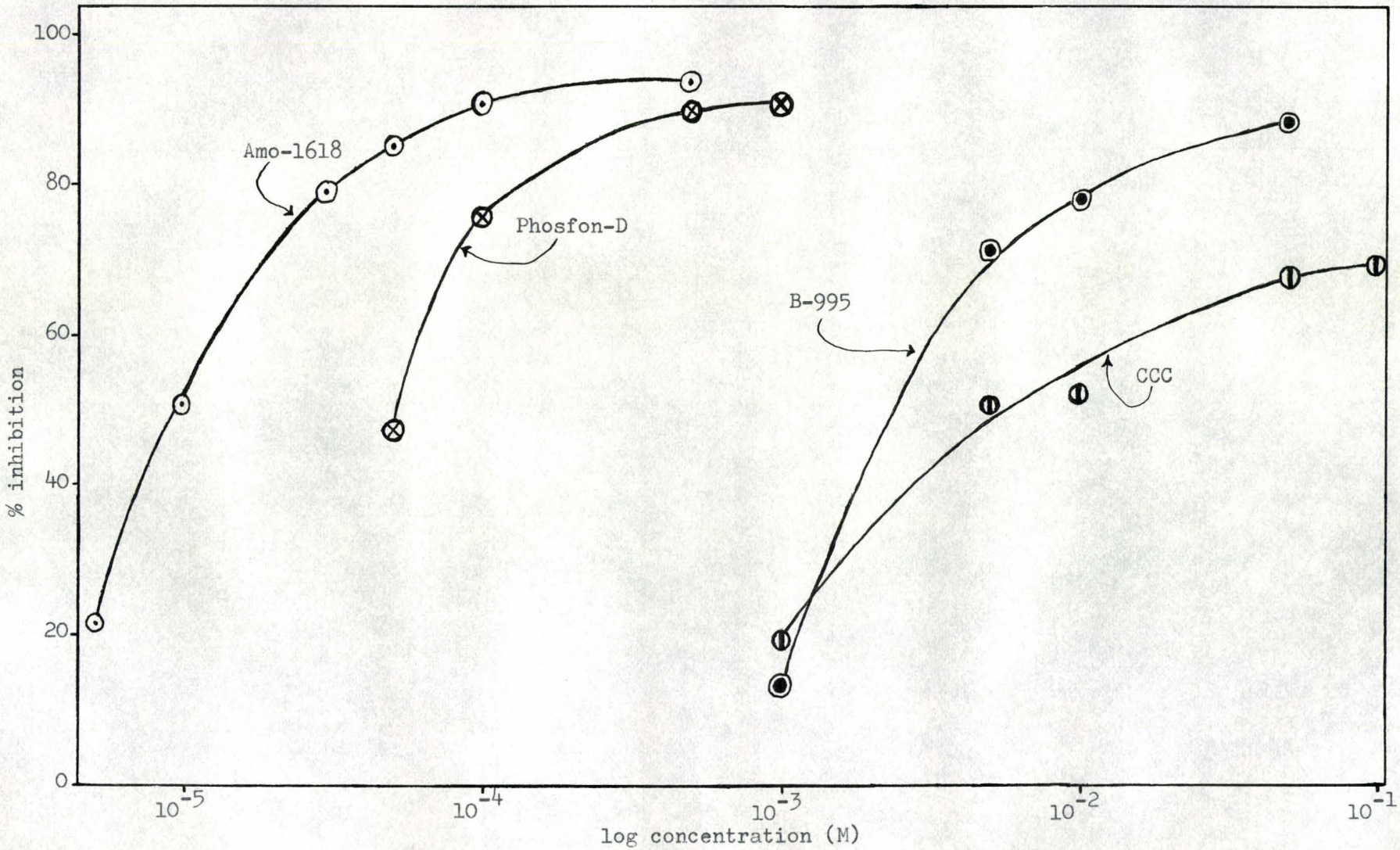


Figure 3. Response of Phaseolus vulgaris to four growth retardants. Total stem length above primary leaves measured after 14 days.

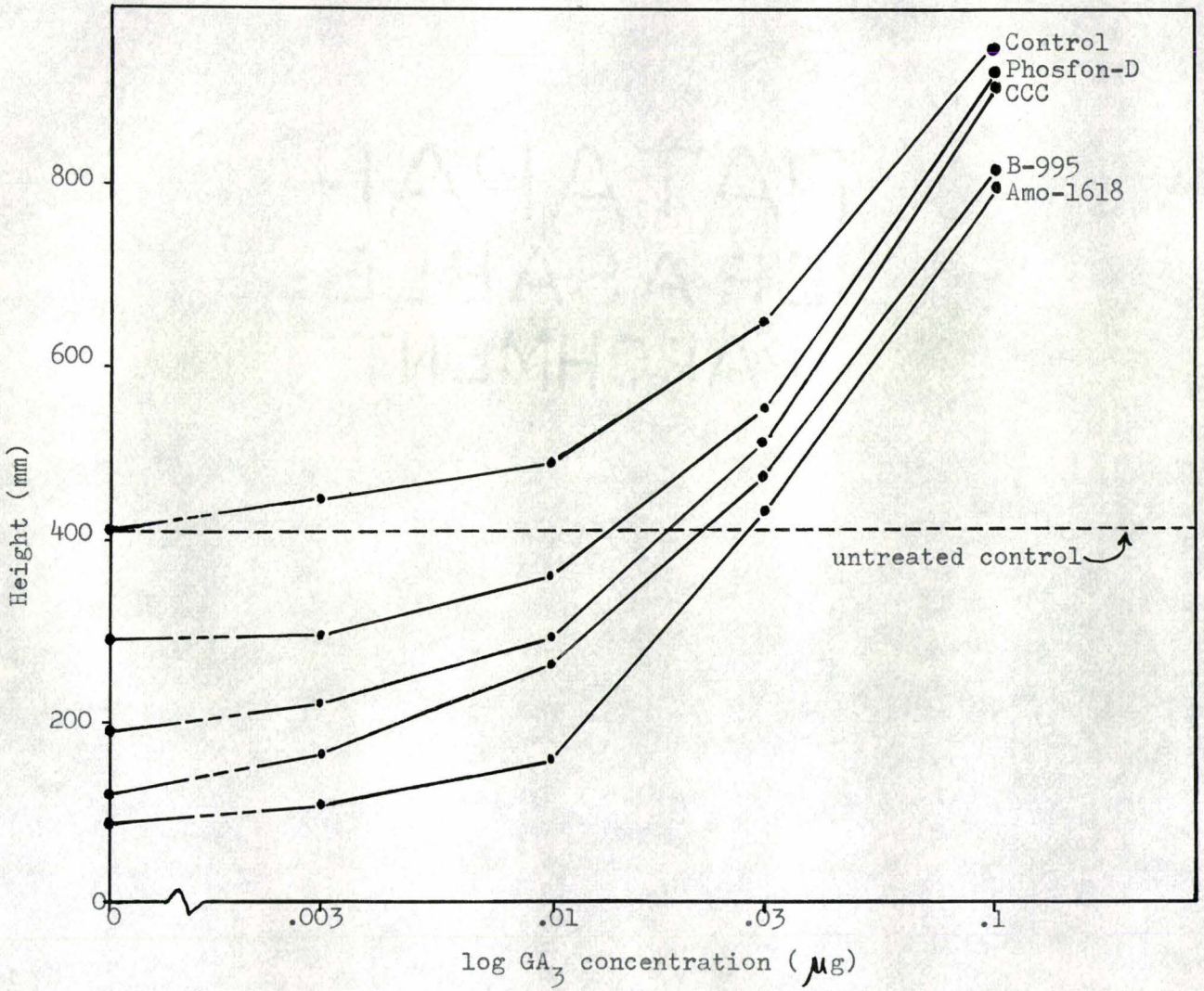


Figure 4. Reversal of growth-retardant induced inhibition by GA_3. Total stem length above primary leaves measured after 14 days.

From the graphs it can be seen that, although various levels of growth retardation were induced, increasing levels of GA_3 caused stem elongation to converge in all cases towards a maximum level approximately equal to that of the treated control plants. Also, in all cases, 0.03μg GA_3 caused a stem elongation greater than that in the untreated controls. This was the concentration of GA_3 chosen for the next set of experiments, since it gave a measurable result which was still below the saturation level.

(ii) Effect of time of treatment with GA_3. In order to determine if GA_3, when applied later than the growth retardant, can still reverse its effect, plants treated with growth retardant were given 0.03μg GA_3 at 0, 5, and 10 days after the growth retardant treatment. Figure 5 shows the effect of the GA_3 treatment alone on control plants. The same rate of stem elongation was caused by the GA_3 whether it was applied after 5 or 10 days, or at day 0. Plants which were treated on day 5 reached the same height as those treated on day 0; those treated on day 10 were slightly shorter.

Figures 6-9 show the effect of similar GA_3 treatments on plants treated at day 0 with Amo-1618 (10^{-5}M), Phosfon-D (10^{-4}M), B-995 ($5x10^{-3}$M) and CCC ($5x10^{-2}$M) respectively. For all four chemicals, GA_3 caused some reversal of the retardation whether applied on the same day as the growth retardant, or later. However, the rate of growth caused by the GA_3 was considerably less when this treatment was given on the 10th day, and the response was not evident until at least 5 days after the treatments as compared to 2-3 days for treatment on the fifth day. The average final height of these plants was in all 4 cases much less

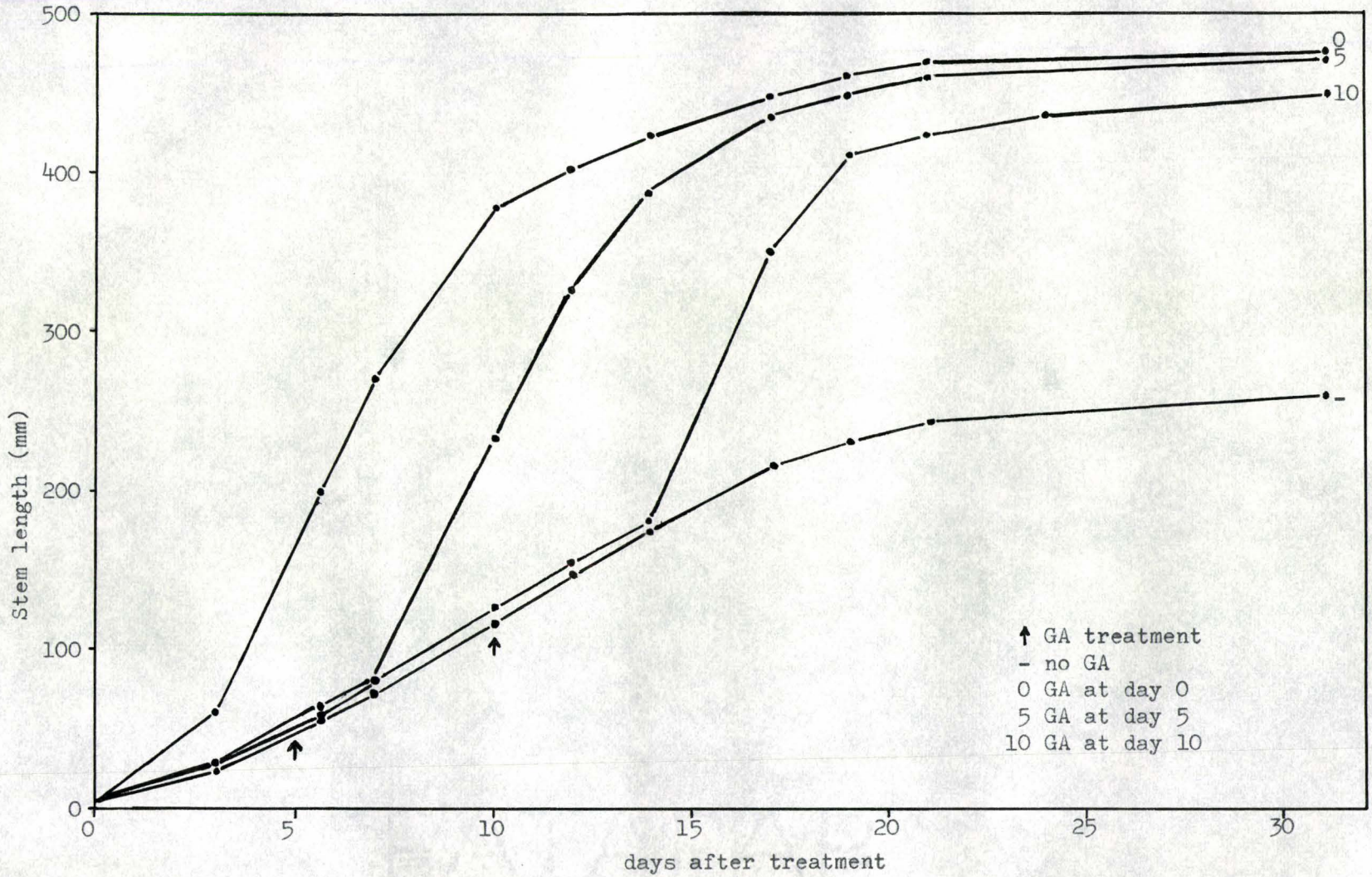


Figure 5. Effect of 0.03 µg GA_3 on plants of P.vulgaris. Total stem length measured above primary leaves.

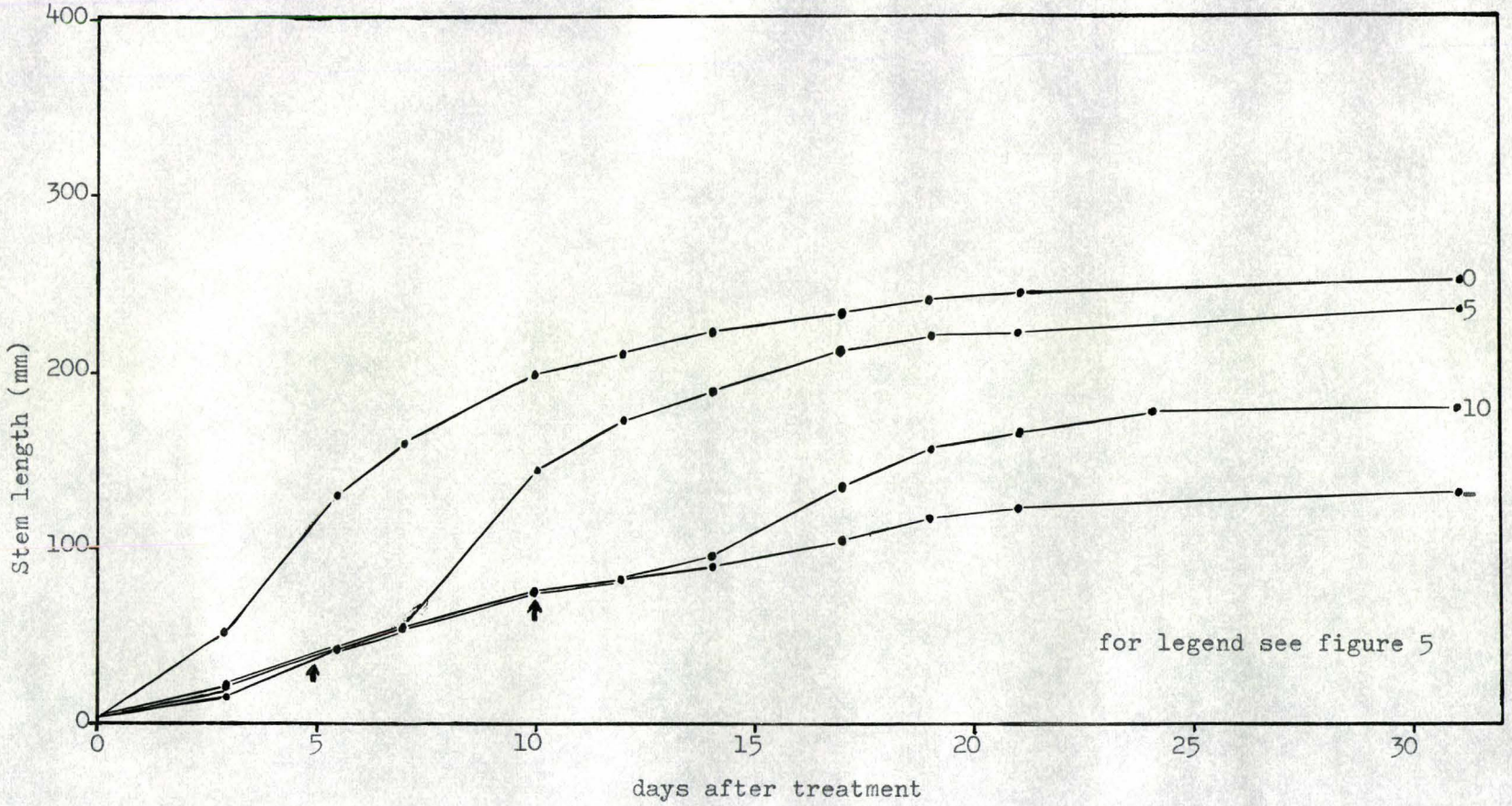


Figure 6. Effect of 0.03 µg GA_3 on plants of P.vulgaris treated with 10^{-5}M Amo-1618 at day 0. Stem length measured above primary leaves.

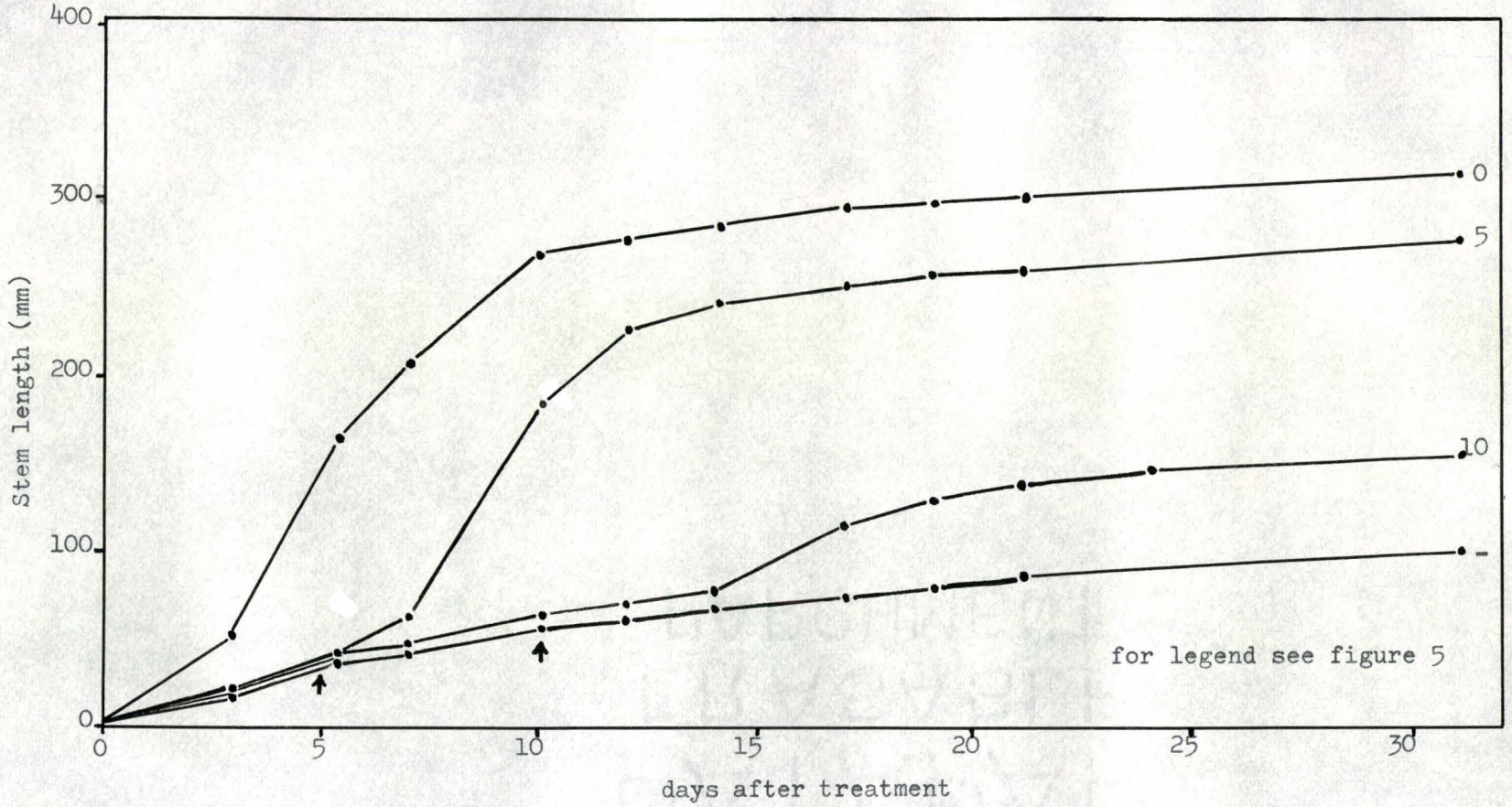


Figure 7. Effect of 0.03 µg GA_3 on plants of P.vulgaris treated with 10^{-4}M Phosfon-D at day 0. Stem length measured above primary leaves.

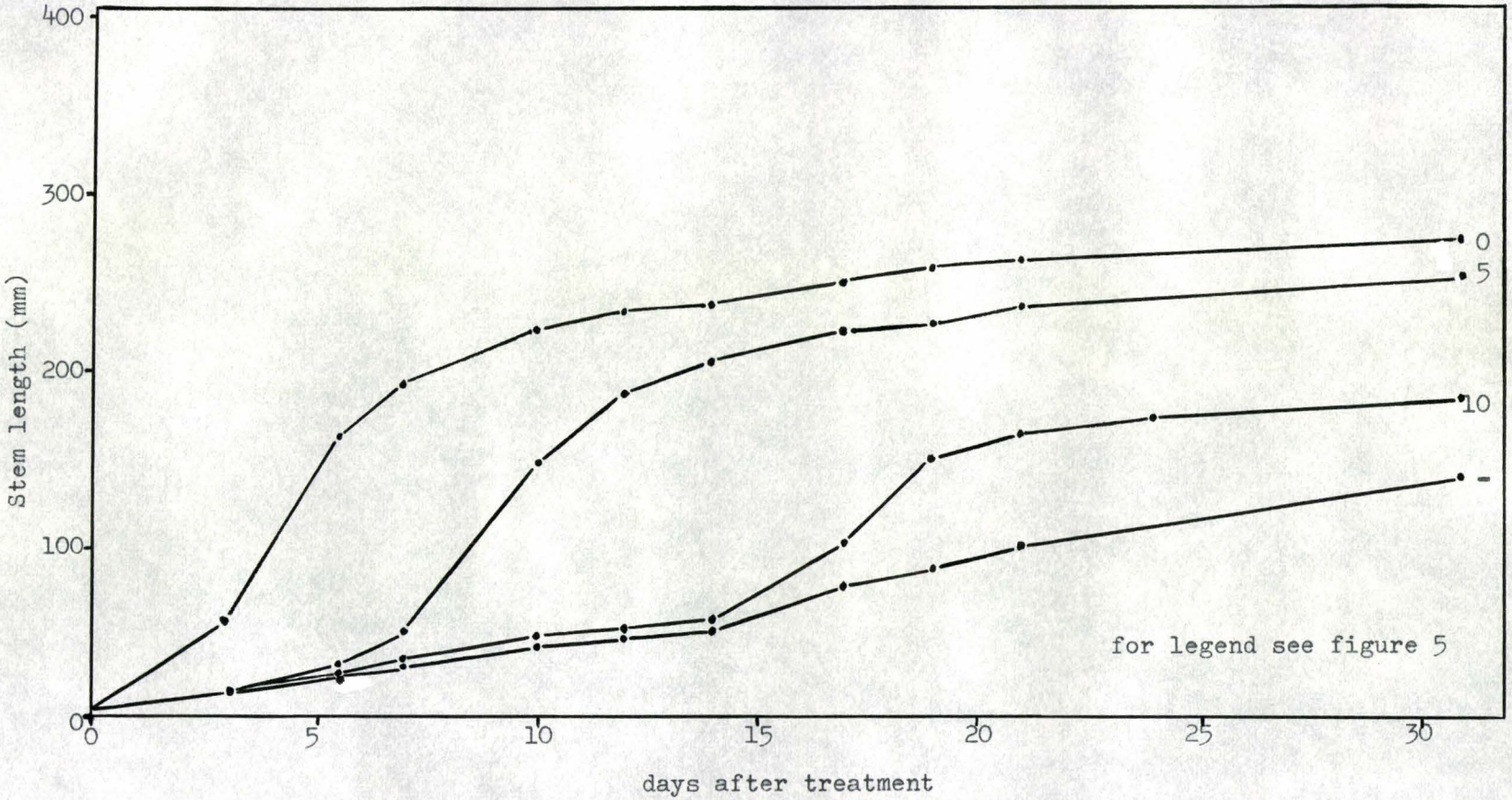


Figure 8. Effect of 0.03 µg GA_3 on plants of P.vulgaris treated with $5X10^{-3}$M B-995 at day 0. Stem length measured above primary leaves.

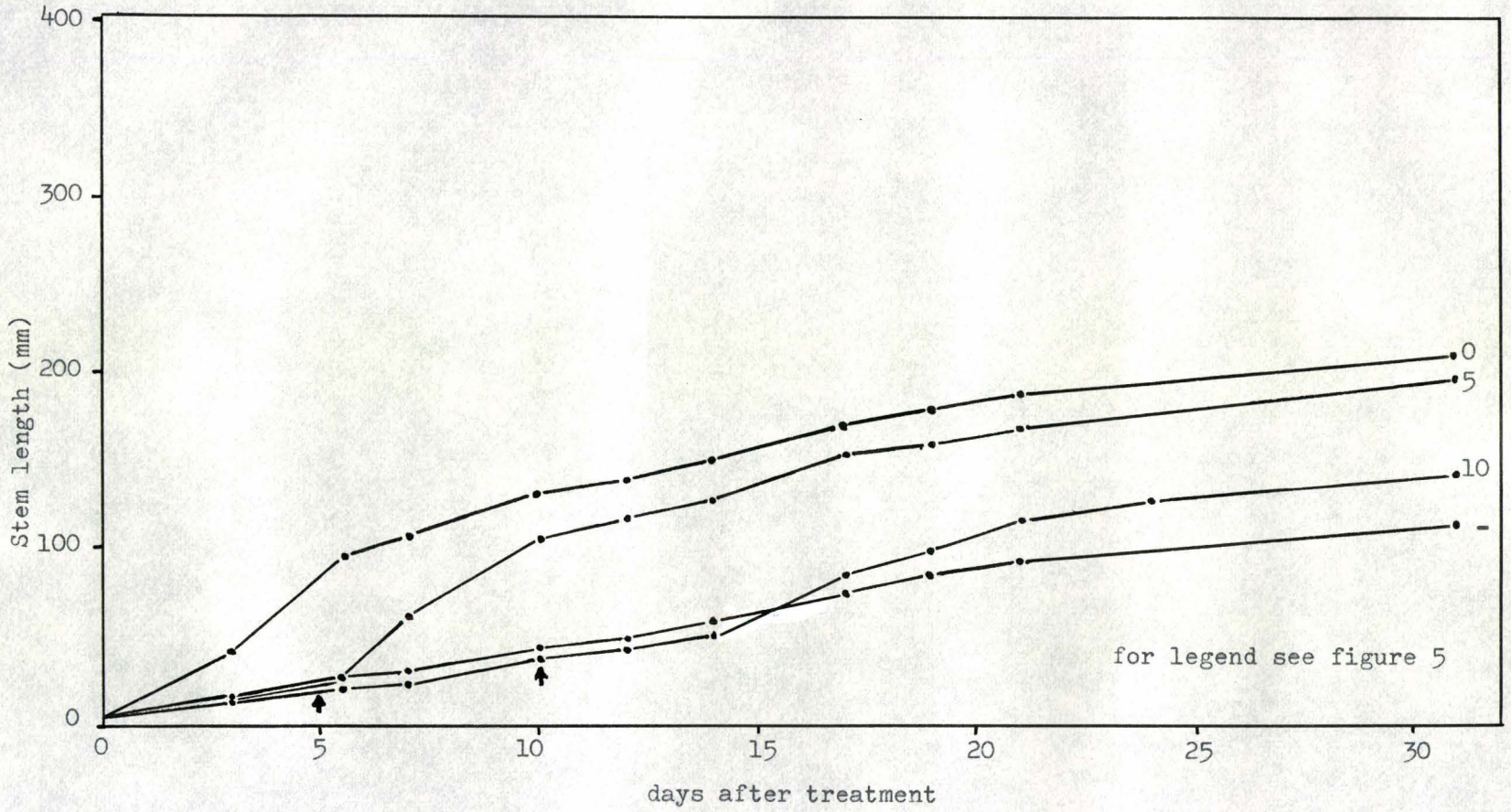


Figure 9. Effect of 0.03 µg GA_3 on plants of P.vulgaris treated with $5X10^{-2}$M CCC at day 0. Stem length measured above primary leaves.

than that of plants treated with GA_3 on day 0 or day 5 (see also figure 10).

Phosfon-D, which caused the most inhibition of growth at the concentration used, still showed the greatest response to GA treatment. CCC-treated plants showed the least recovery with GA, although plants treated with CCC and B-995 (especially the latter) appeared to be "growing out" of the retardation after 14-15 days. This spontaneous recovery is known to occur in Pharbitis nil where the effectiveness of a growth retardant can be measured by the number of days maximal growth rate is delayed (Zeevaart, 1964).

The final results are summarized in figure 10. The response to GA_3 of plants treated with the four chemicals can be compared to the response of untreated plants to GA_3. It is obvious that retarded plants do not respond as well to the later treatments with GA_3, especially if they are given as late as day 10. The greater sensitivity of Phosfon-D treated plants to GA_3 can readily be seen here also.

In this experiment, flowering began 17 days after application of growth retardants. An earlier experiment of the same type with GA_3 treatments on day 8 and day 16 gave similar results, although flowering interfered with the response to the later treatment by causing termination of extension growth. This effect of flowering does not, however, explain the relatively lower sensitivity of the retarded plants, compared to control plants, to GA_3 treatment on day 10. Termination of extension growth due to flowering would occur earlier in control plants, since flowering is delayed in treated plants (Preston, 1961). This would cause control plants to lose sensitivity to GA_3

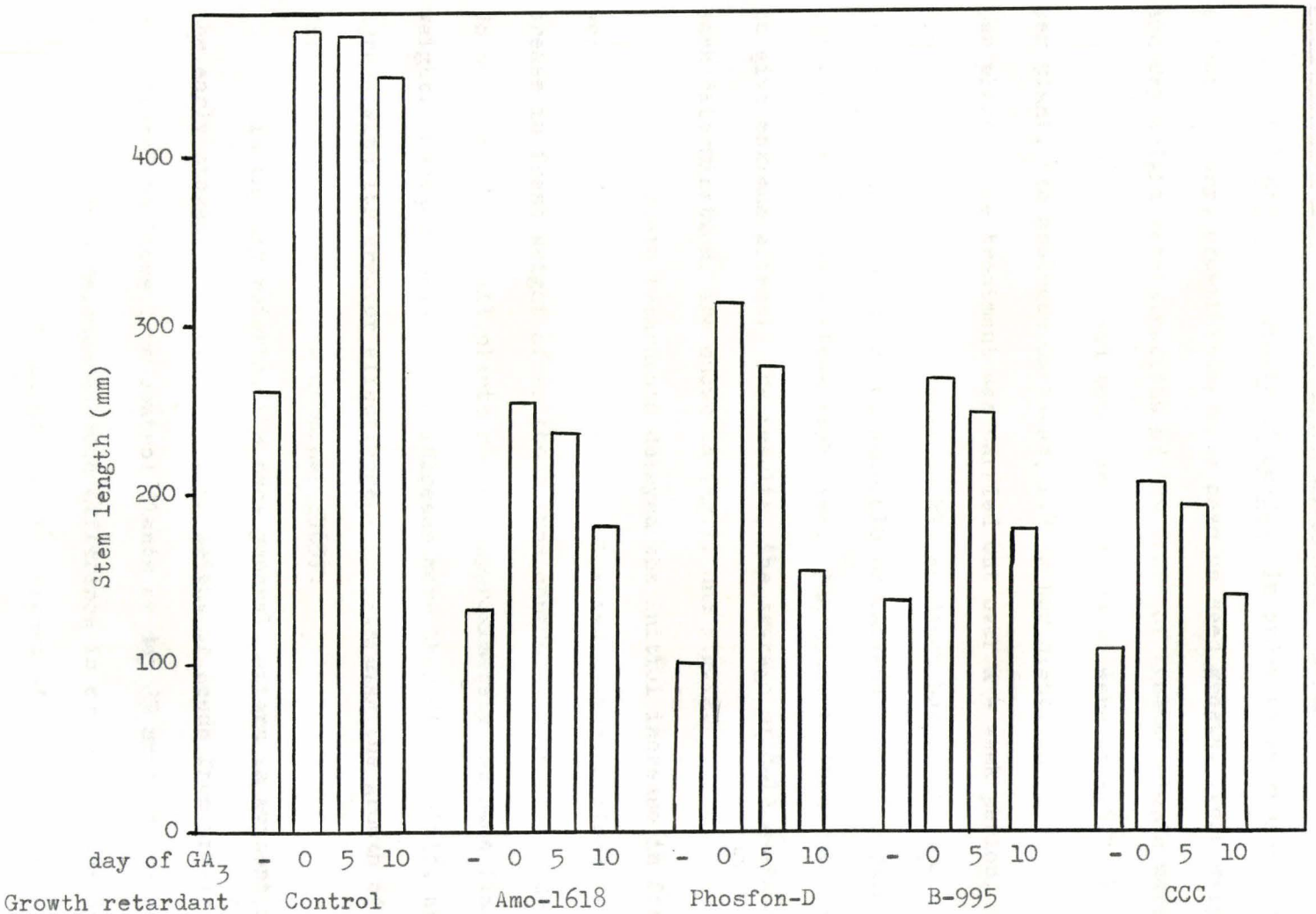


Figure 10. Total stem length after 31 days of plants of P.vulgaris treated with growth retardant and GA_3.

before treated plants, and the effect would be less response in control plants than in retarded plants.

3. Effect of growth retardants on growth in progeny

(i) Effect on growth of seeds. In order to determine what effect, if any, growth retardants have on seed growth, fresh weight and dry weight determinations of the fruit of treated plants were made.

The chemicals and total amounts tested were Amo-1618, 1.4 mg per plant, its analogue Carvadan, 1.4 mg per plant, and CCC, 190 mg per plant. The treatment was carried out over a 4 week period, the chemicals applied via the roots weekly as described in ch.II p.9. Ten ml of a 10^{-4}M solution of Amo-1618 or Carvadan, or of a $3x10^{-2}$M solution of CCC, were given each time. The concentrations were chosen to give maximum effect. The results, the average of 8-12 seeds for each determination, are shown in fig.11 and fig.12.

The growth retardants delayed the initial increase in fresh weight (fig.11), but seeds from these treated plants continued to increase in fresh weight after those from control plants had ceased to do so. Seeds from all plants reached approximately the same final weight. Carvadan delayed the increase more than did Amo-1618, which agrees with its greater effectiveness in retarding the growth of intact plants (Zeevaart and Osborne, 1965).

In the dry weights, the same general pattern is evident in the early stages. The greater fresh weight of seeds from treated plants as compared to those from control plants at day 25 must be due to greater water content, because no such difference is evident in the dry weight. At maturity (30 days) the dry weights of all seeds are

Figure 11

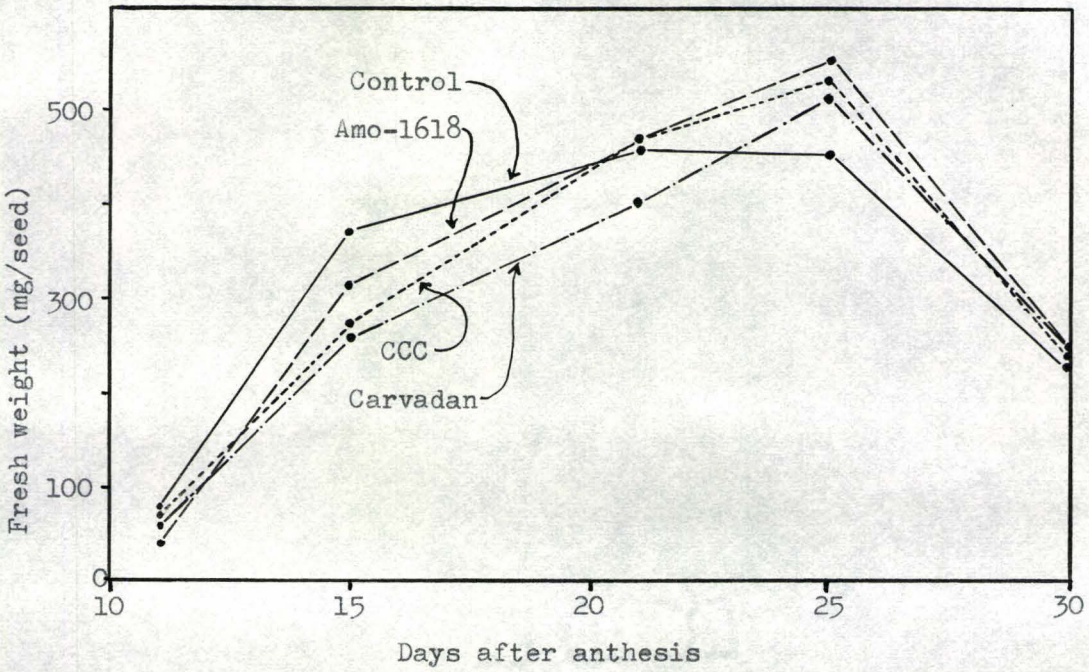
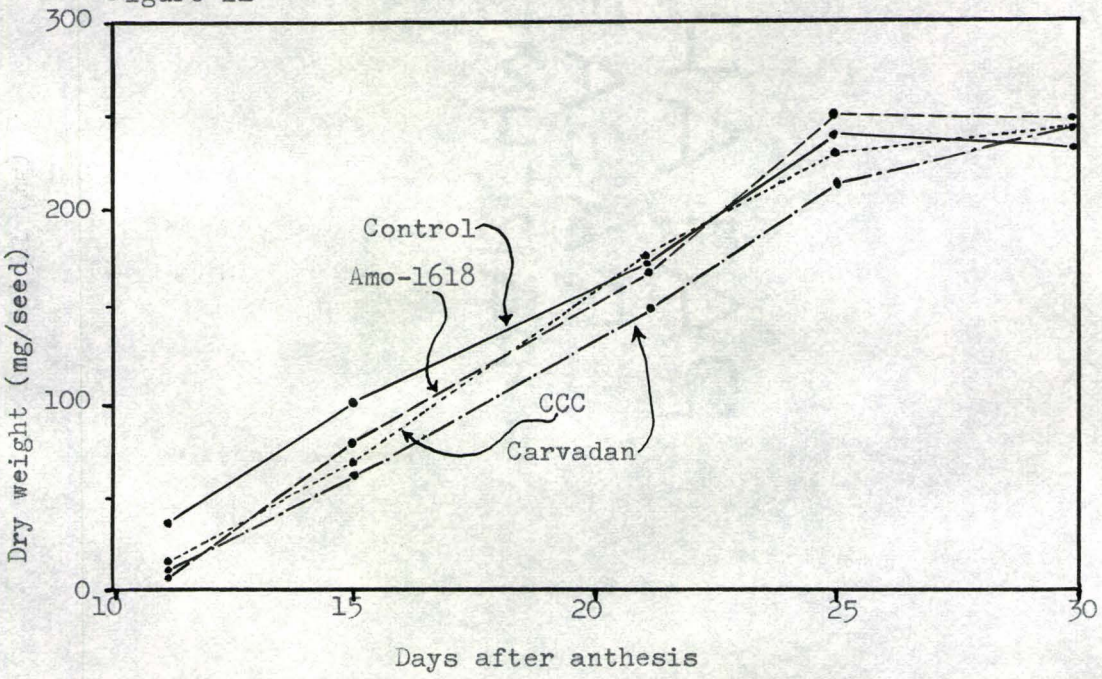


Figure 12



Figures 11, 12. Fresh weights and dry weights of P_1 progeny from plants of P.vulgaris treated with growth retardants.

approximately the same.

(ii) Effect on stem elongation in P_1, P_2, and P_3. Mature seeds were collected from the first progeny (P_1), second progeny (P_2) and third progeny (P_3) of treated plants. Ten of the largest seeds of each progeny were selected from each treatment and weighed before planting. The weights are recorded below:

Table 1. Weight of 10 Seeds planted per treatment.

Treatment	Progeny	Wt. of 10 seeds (mg)
Control	P_1	2987
	P_2	2323
	P_3	2675
CCC (total of 200 mg in 5 doses)	P_1	2865
	P_2	2407
	P_3	2686
Carvadan (total of 50 mg in 5 doses)	P_1	2832
	P_2	2598
	P_3	2493

Seeds of the P_1 generation only were collected in a second experiment where smaller amounts of growth retardant were given. The weights of the ten seeds selected from each treatment are given below:

Treatment	Weight of 10 seeds (mg)
Control	2371
CCC (24 mg, 4 doses)	2542
Amo-1618 (1.4 mg, 4 doses)	2406
Carvadan (1.4 mg, 4 doses)	2416

The seeds were planted, one per container (950 ml) and grown as described in ch.II p.7. The results are shown in plates 1-4 and summarized in figure 13. The progeny from the first experiment is labelled "I", that from the second experiment "II".

Plate 1 shows plants of average height taken from P_1, P_2 and P_3 in the control group. The parent plants here had no treatment except 10 ml of water when treated plants received 10 ml of growth retardant. However, the P_2 and P_3 generations are somewhat shorter than the P_1. This difference reflects relatively poor seed set obtained while growing plants for the P_2 and P_3 progeny.

Plates 2 and 3 show the progeny from plants treated with CCC (200 mg) and Carvadan (50 mg). The height of these plants is expressed as a % of the corresponding control progeny in figure 13. The retardation of growth in the P_1 generation is evidence of the transfer of dwarfing response from the parent plant, treated during anthesis, to the progeny. The growth of the P_1 progeny from plants treated with CCC was inhibited by 68% (fig.13). The progeny of this generation, P_2, and the next, P_3, showed no effect of the growth retardant on stem elongation, and exhibited normal growth.

The first progeny of plants treated with Carvadan showed 99% inhibition of stem elongation. The progeny of these plants was 70% inhibited. The P_3 generation exhibited normal growth.

When lower concentrations of the growth retardants were used, proportionately less inhibition was observed in the P_1 generation (plate 4; fig.13). Amo-1618 was less effective than Carvadan in causing dwarfing of progeny,as would be expected considering its lower

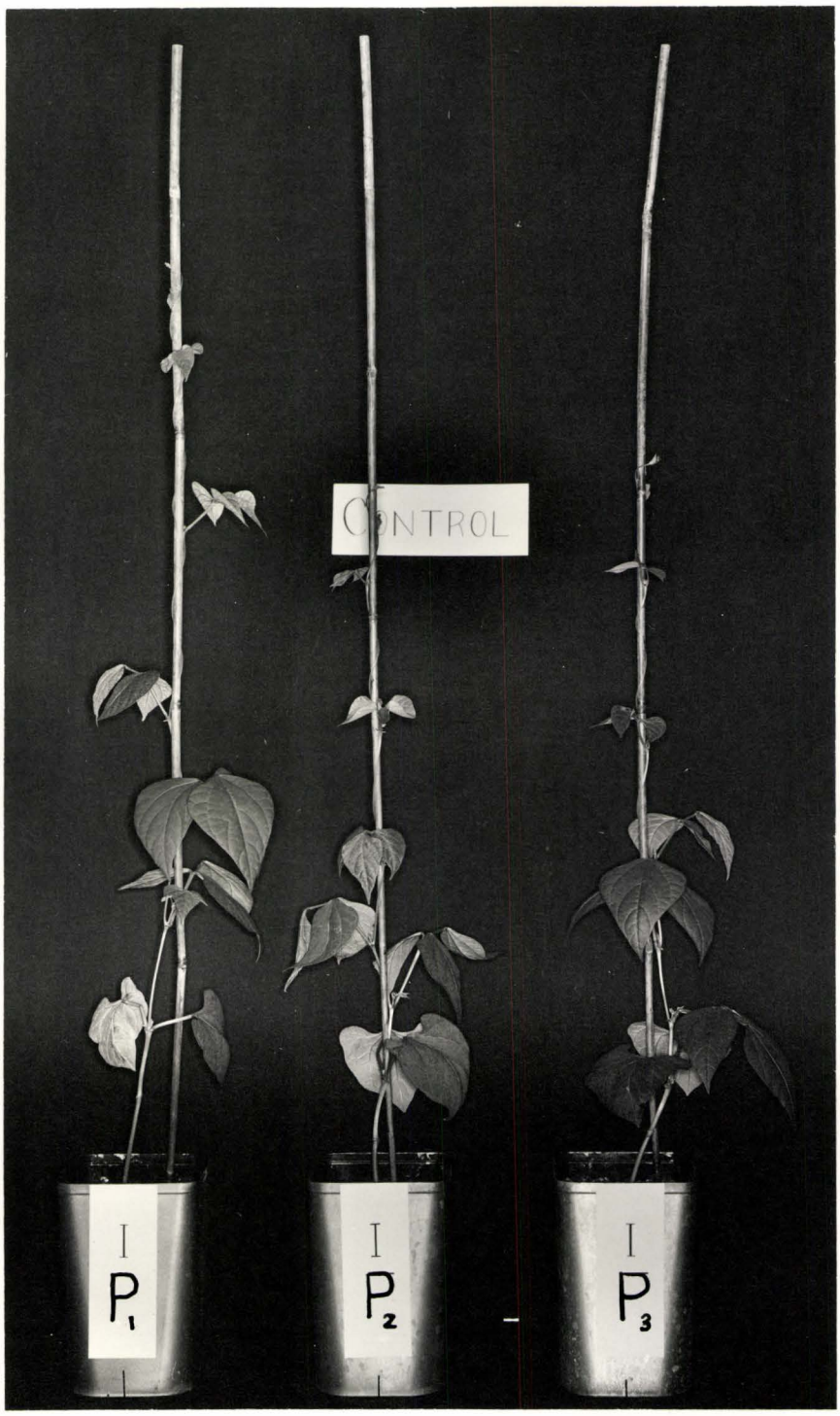


Plate 1. First, second and third progeny of Control plants from Experiment I.

Plate 2. First, second and third progeny of plants treated with 200 mg CCC--Experiment I.

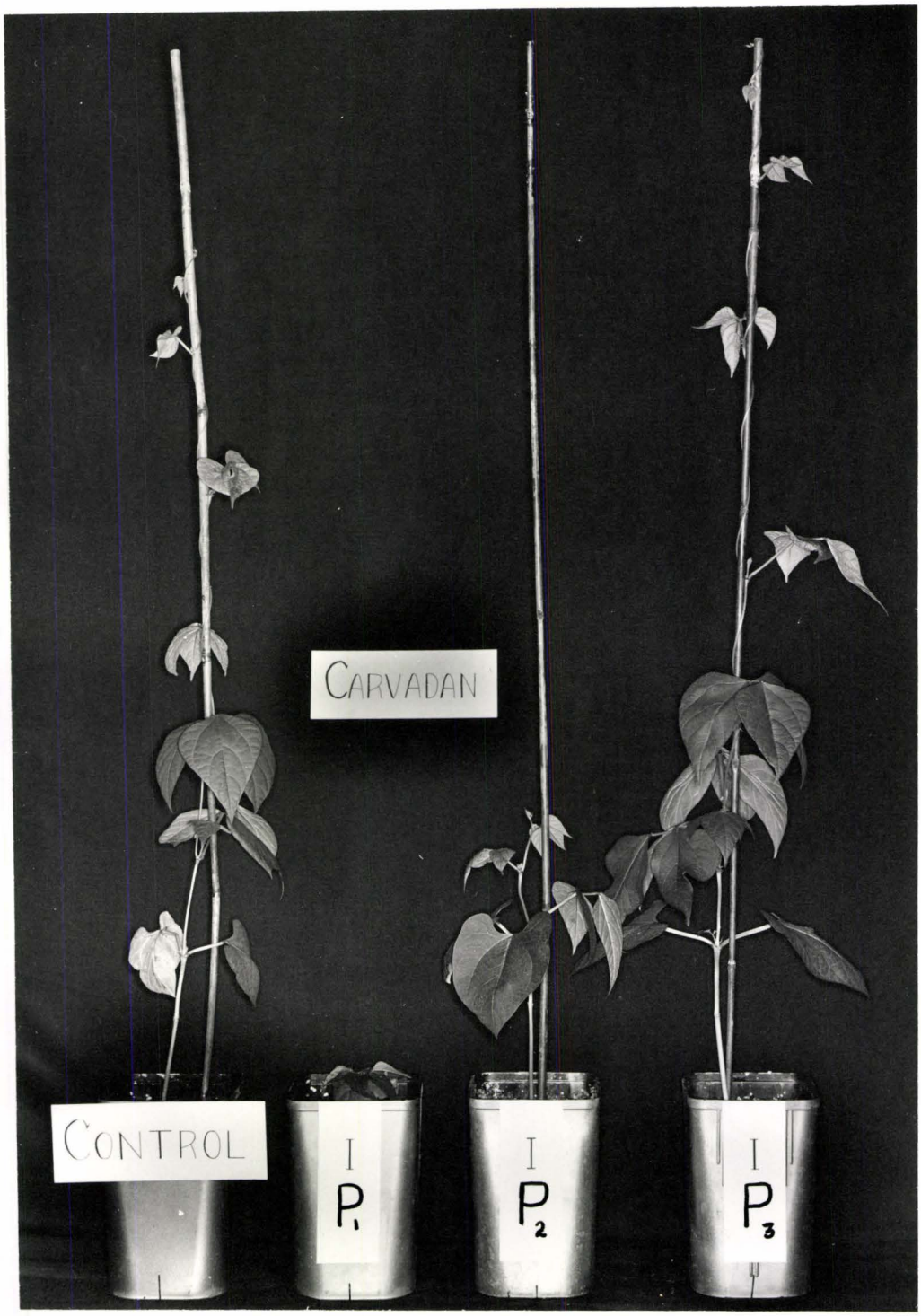


Plate 3. First, second and third progeny of plants treated with 50 mg Carvadan--Experiment I.

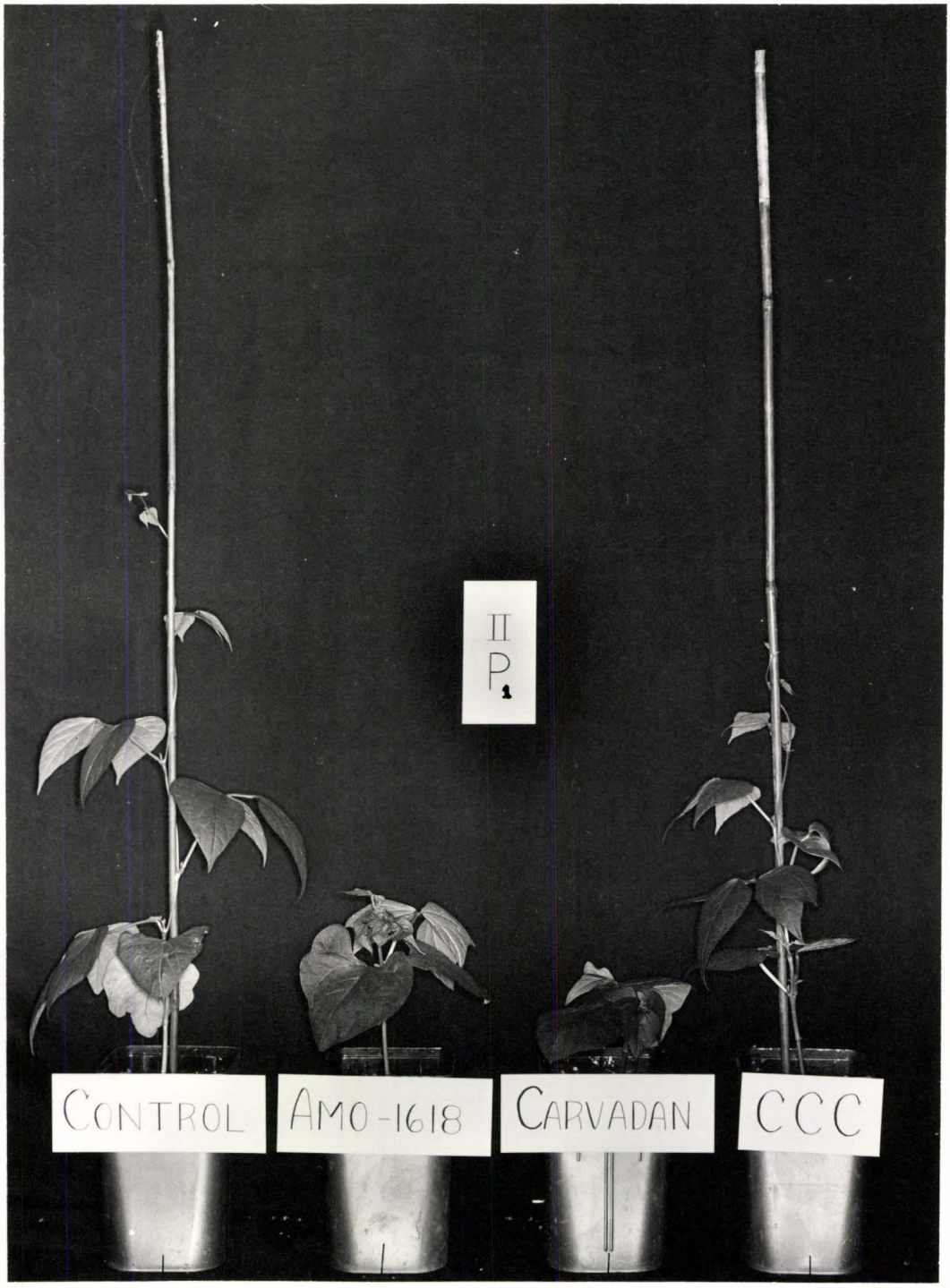


Plate 4. First progeny of plants treated with 1.4 mg Amo-1618, 1.4 mg Carvadan, or 2 mg CCC--Experiment II.

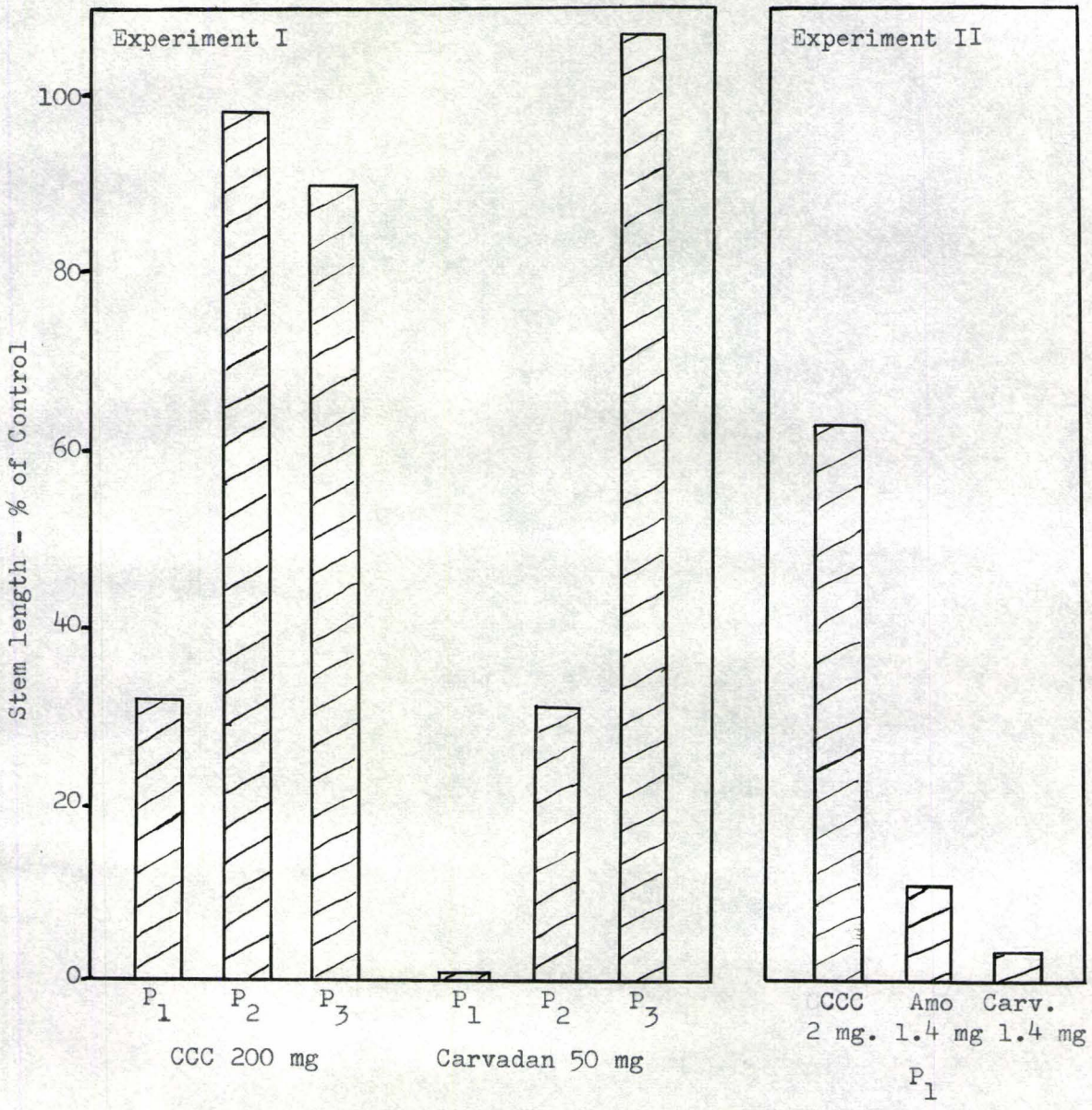


Figure 13. Stem growth in progeny of plants treated with growth retardants. Total stem length above primary leaves measured after 14 days.

P_1: first progeny

P_2: second progeny (progeny of P_1)

P_3: third progeny (progeny of P_2)

effectiveness on stem elongation (Zeevaart and Osborne, 1965).

(iii) Reversal of dwarfing in progeny. To test whether the dwarfing response was due to toxicity of the growth retardants, or whether inhibition was brought about through a similar mechanism to that in plants treated with growth retardant via the roots, GA_3 was applied to the primary leaves of young plants from the P_1 progeny of plants treated with 50 mg Carvadan. Control plants were also treated, using methods for GA reversal experiments described in chapter II. The results are shown in figure 14. GA_3 reversed the dwarfing response in progeny as much as it reversed the inhibition caused by 15 mg Amo-1618 applied via the roots. This demonstrates that growth inhibition in the progeny of treated plants is not due to toxicity of the chemicals, and is probably brought about by the action of the chemicals themselves through the same GA-reversible mechanism as in plants treated directly with the growth retardants. To show this, it is necessary to demonstrate the presence of the growth retardants in the seeds, in amounts sufficient to cause the observed response.

Figure 14. Reversal of dwarfing by GA_3 in P_1 progeny compared to reversal in treated plants. Stem length above primary leaves measured after 9 days.

CHAPTER IV

Extraction of Seeds for GA-like Substances and Growth Retardants

1. The nature of GA's present in immature seeds

In order to determine which gibberellins are present in pinto beans, 300 immature seeds were collected from plants grown in the field. The fresh weight per seed was 460 mg; dry weight per seed after lyophilization was 149 mg. Twenty seeds were chosen at random and the length measured. The length varied from 12 to 15 mm; the average length was 13.1 mm.

(i) Thin-Layer Chromatography. The 300 seeds were extracted as described in ch.II p.13 and the acidic fraction obtained was separated into 10 fractions using column chromatography (ch.II,p.13). Each of the ten fractions was dissolved in 1.0 ml ethyl acetate and TLC was carried out on Silica gel H (250μ) in three solvent systems. The plates were run for 15 cm, sprayed with ethanolic H_2SO_4 and heated for 10 minutes at 120°C, then viewed under U.V. light. The results are compared with R_f values of the known GA's reported in the literature in Table II.

Two spots, designated GA_x and GA_y, were visible under U.V. light in fraction 9, and in fraction 8, where they were fainter. GA_x, visible in systems I and III, had the same R_f value and colour of fluorescence as the GA_1-standard, and was tentatively identified as GA_1. This identification is supported by the results of Kende

Table 2. Observed and reported R_f values for GA's in 3 solvent systems.

GA	SOLVENT SYSTEM					
	I[1]		II[2]		III[3]	
	R_f obs.	R_f rep.	R_{GA_3} obs.	R_{GA_3} rep.	R_f obs.	R_f rep.[4]
GA_1	.17	.11	.98	1.0	.15	.39
GA_3	.13	.11	1.0	1.0	.15	.35
GA_4	.57	.37	1.11	1.17	.48	.74
GA_5	.54	.31	.98	1.0	.45	.74
GA_8	---	.04	---	.89	---	.21
GA_9	.95	.75	1.11	1.19	.80	.88
GA_x	.17		---		.15	
GA_y	.04		.92		.06	

[1] di-isopropyl ether/acetic acid (95:5) (MacMillan and Suter, 1963)

[2] isopropanol/water (4:1) (Elson et al, 1964)

[3] chloroform/ethyl acetate/acetic acid (60:40:5)(Sembdner et al, 1962)

[4] elution chromatography

and Lang (1964). They found GA_1 to be present in the sixth and seventh of 8 fractions (compared to the eighth and ninth of 10 fractions, above) obtained by column chromatography of dwarf pea extracts.

GA_y can also be identified with some certainty, as GA_8. The R_f or R_{GA_3} values observed for GA_y in systems I and II agree very closely with the reported value for GA_8. In system III, as in the other two systems, the R_f of GA_y was slightly less than that of GA_1. This would be expected if GA_y were GA_8, since the latter is more hydrophilic than GA_1.

The high R_f value of GA_y in system II, and the relative positions of GA_x and GA_y in the other two systems, indicate that GA_x probably ran in the solvent front or just behind it in system II, and hence could not be seen.

No fluorescent spot was visible in any of the fractions which would correspond to GA_5, although this GA has been reported to be present in Phaseolus vulgaris (West and Phinney, 1959). Kende and Lang (1964) found a GA probably identical with GA_5 in the second and third of eight fractions collected by column chromatography of dwarf pea extracts. In both cases, bio-assays were used to detect this GA, since chromatography is less sensitive.

(ii) Bio-assays.

Bio-assays, which give a more sensitive means of detecting gibberellins, were also used. The results are shown in figure 15 for bio-assays of the ten fractions on dwarf pea, dwarf corn d-5, and dwarf corn d-1.

Fractions 2 and 3 were inactive on dwarf pea and on d-1 corn,

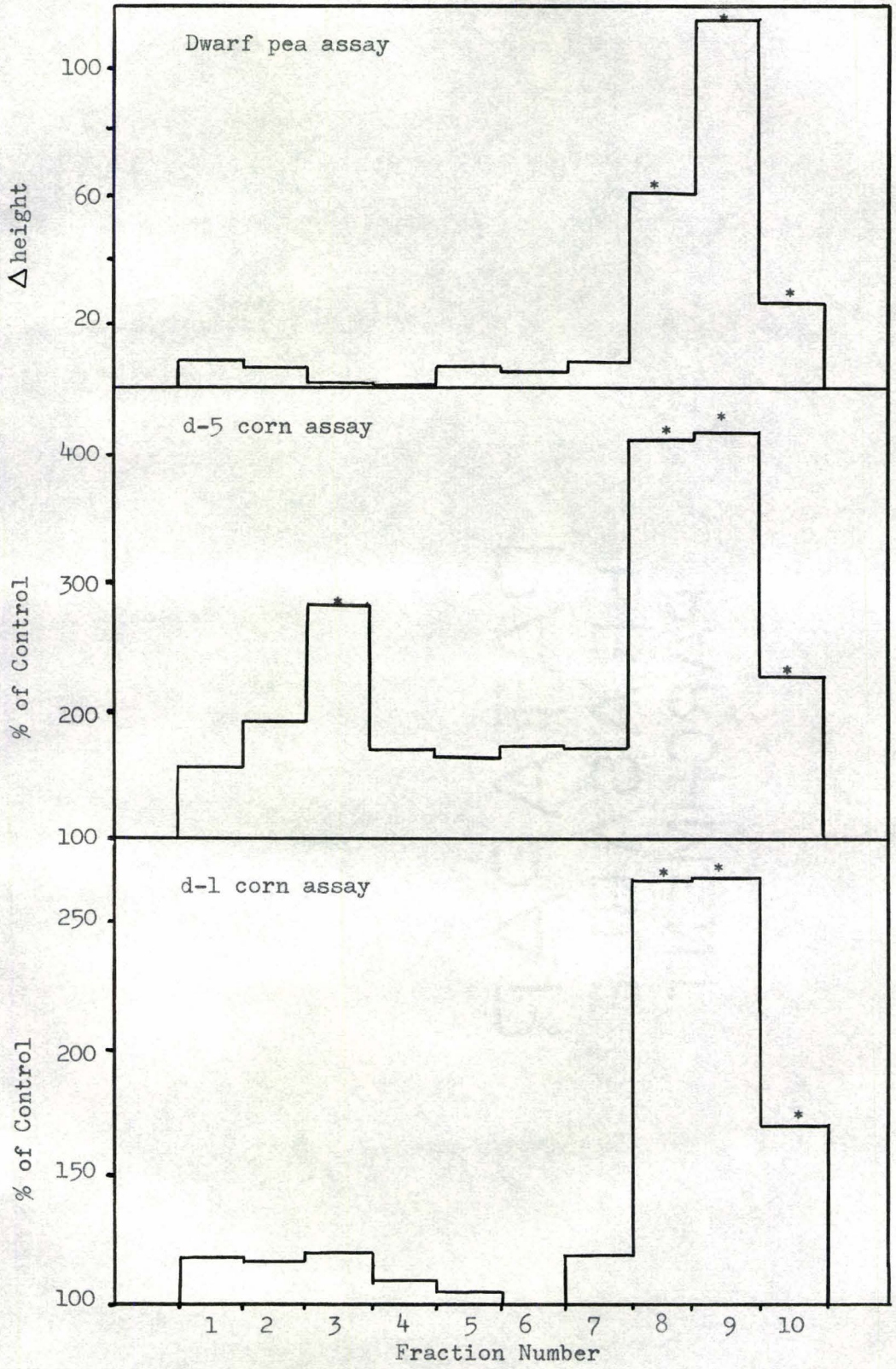


Figure 15. Bio-assays of 10 fractions from column chromatography of extracts of immature seeds of P.vulgaris.

but active on d-5. These results, supported by those of Kende and Lang mentioned above, are good evidence for the presence of GA_5 in these fractions, since GA_5 is highly active on d-5 corn but only moderately active on dwarf pea and d-1 corn (Thimann, 1963).

Since the amounts of fractions 8 and 9 applied gave saturation levels of increased height on both dwarf corn bio-assays, the results of the dwarf pea test were used to give the following quantitative estimates of the amounts of GA present in these fractions:

fraction 8: 9.8 μg GA_3-equivalents/1000 seeds

fraction 9: 67.3 μg GA_3-equivalents/1000 seeds

Since GA_8 is inactive in all systems (Brian et al, 1964), the response is probably due mainly to GA_1.

From the dwarf corn d-5 bio-assay, the μg's GA_3 equivalents in fractions 2 and 3, which probably contain GA_5, and the minimum amounts for fractions 8 and 9, are:

fraction	μg GA_3-equivalents/1000 seeds
2	0.8
3	11
8	20
9	20

Twenty μg GA_3-equivalents represents the saturation level at the sample dilutions used for this bio-assay. Since d-5 corn is more sensitive to GA_1 than is dwarf pea (Brian et al, 1964), a higher level of GA-activity was observed in fraction 8 using this test.

2. Effect of Growth Retardants on GA Content of Immature Seeds

Gibberellins were extracted from immature seeds of plants

treated with 1.4 mg Amo-1618 or Carvadan, or 2 mg CCC (ch.II p.12) and separated using preparative TLC. The solvent system used was di-isopropyl ether/acetic acid (95:5). The GA-activity, determined by dwarf pea bio-assay, of the 10 fractions obtained from an extract of seeds of control plants harvested 15 d. after anthesis is shown in figure 16. Activity was found in bands 1 and 2, probably due to the presence of GA_1, and in band 5, probably due to the presence of GA_5.

The changes in GA activity in extracts of immature seeds from plants treated with growth retardant as compared to those from control plants are shown in figure 17. The test system used was the dwarf pea bio-assay. The µg's GA_3 in bands 1 and 2 were combined to give the total activity in fraction I. Band 5 is active fraction II. The experiment was repeated 2X, with similar results. Enough GA_5 (assuming active fraction II contains GA_5) was present to be detectable in control seeds from day 15 to day 20. The seeds collected from plants grown outside for the first GA determination must have passed the peak of GA_5-content already.

Eleven days after anthesis, when the seeds are still very small (fig.11 and 12) they have already passed the peak of GA activity in fraction I. This agrees with the results of Skene and Carr (1961), who found a similar trend in GA activity in zone 1 of a descending paper chromatogram. Jones (1964) also found differences in the time of appearance and maximum content of the GA's in Phaseolus multiflorus. GA_5 was the first to appear and reach a maximum, followed by GA's 1, 6, and 8. GA_3 appeared later.

The growth retardants have a marked effect on the amount of GA

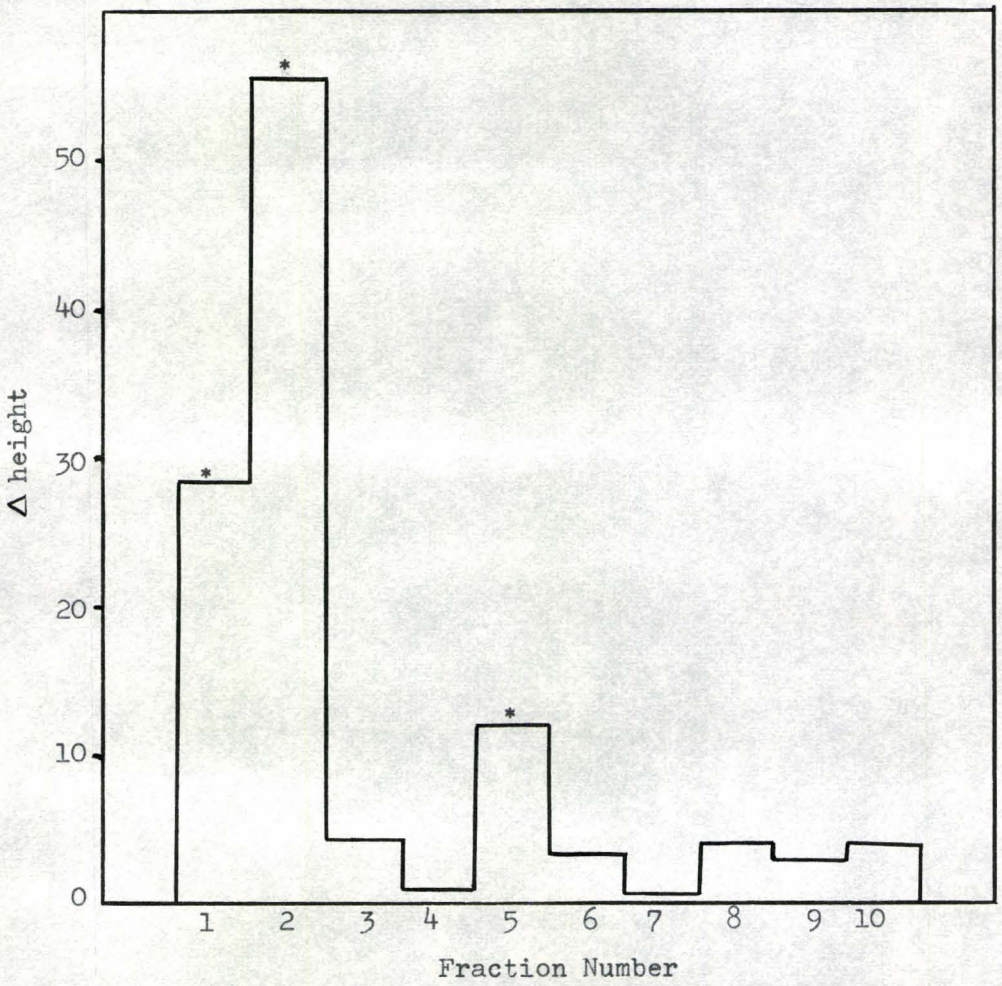


Figure 16. Dwarf pea bio-assay of GA activity in extract of immature seeds (15 d.) of P.vulgaris.

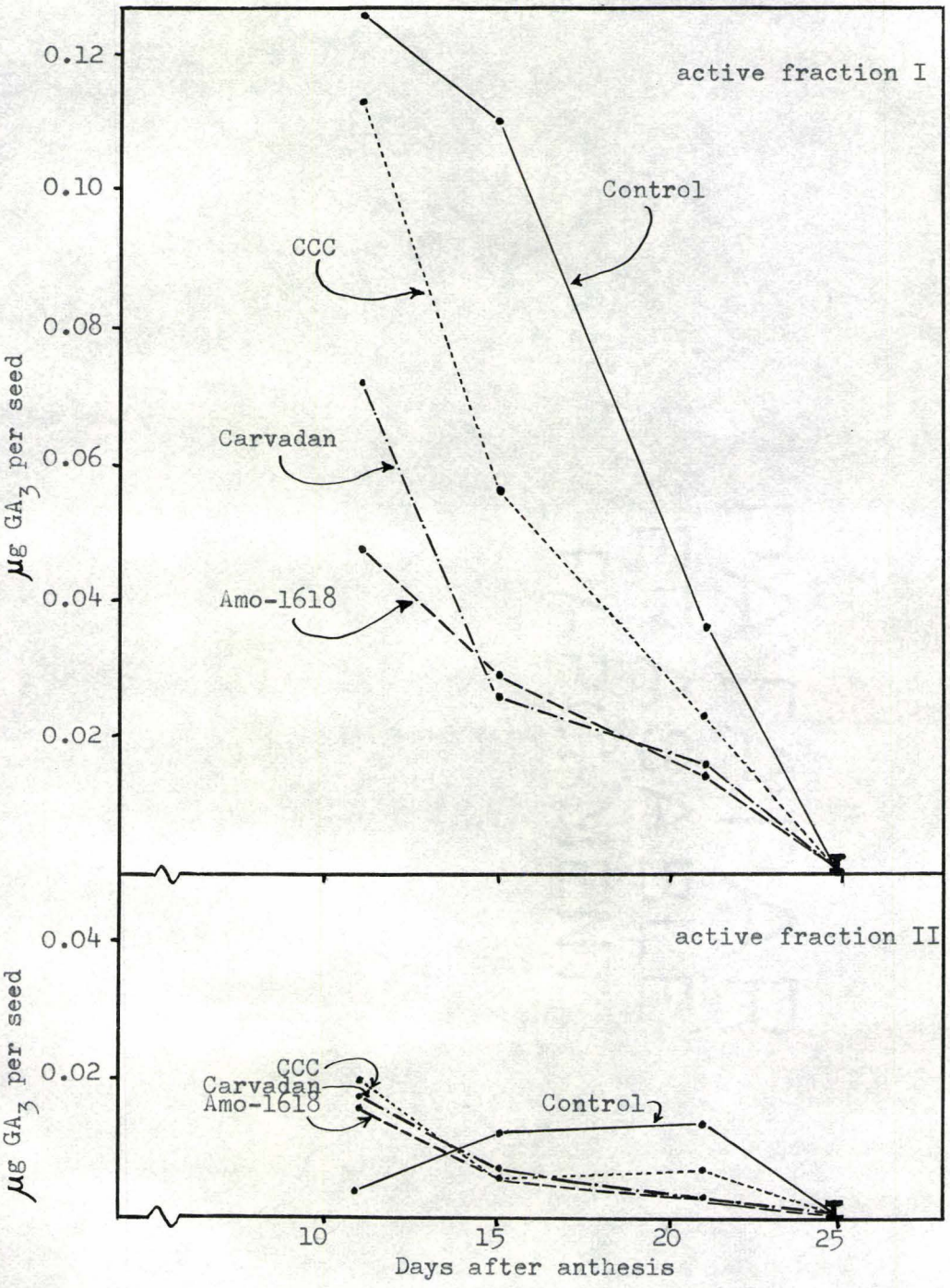


Figure 17. Changes in GA activity in active fractions from immature seeds of plants treated with growth retardant during anthesis, as tested in pea bio-assay.

in fraction I present in the seeds, at the stages measured. CCC, which is least effective in retarding stem elongation in treated plants and in progeny, is also least active in decreasing GA content. Carvadan and Amo-1618 cause a greater decrease in GA content than does CCC. Carvadan is somewhat less effective than Amo-1618. At 25 days after anthesis, no GA activity could be detected in the bio-assays.

The amounts of GA present in fraction II are low at all stages. Here however the trend in the control is different from that in seeds from treated plants. The peak of GA in fraction I in control seeds occurs between 15 and 20 days after anthesis; in seeds from plants treated with Amo-1618 and Carvadan it occurs before 11 days and declines steadily after that time. CCC lies somewhere between the latter two and the control treatment. The peak is reached before 11 days, with a decline until about 15 days. The activity then levels off or rises slightly to a peak which coincides with that in control seeds, although this is probably not significant. At 25 days, no GA is detectable in any of the seeds.

Figure 18 is a comparison of the results from a pea bio-assay and a d-5 corn bio-assay of the active fractions from seeds of treated and untreated plants. No quantitative comparison can be made because of the differing sensitivities of the two bio-assays to GA_3, the standard. In general, the d-5 corn test gives a lower value for total GA-activity than does the dwarf pea test on the same sample. However, the % of control height reached by plants treated with fraction II from Amo-1618 or CCC seeds corresponds to 0.03 μg GA_3-equivalents per seed.

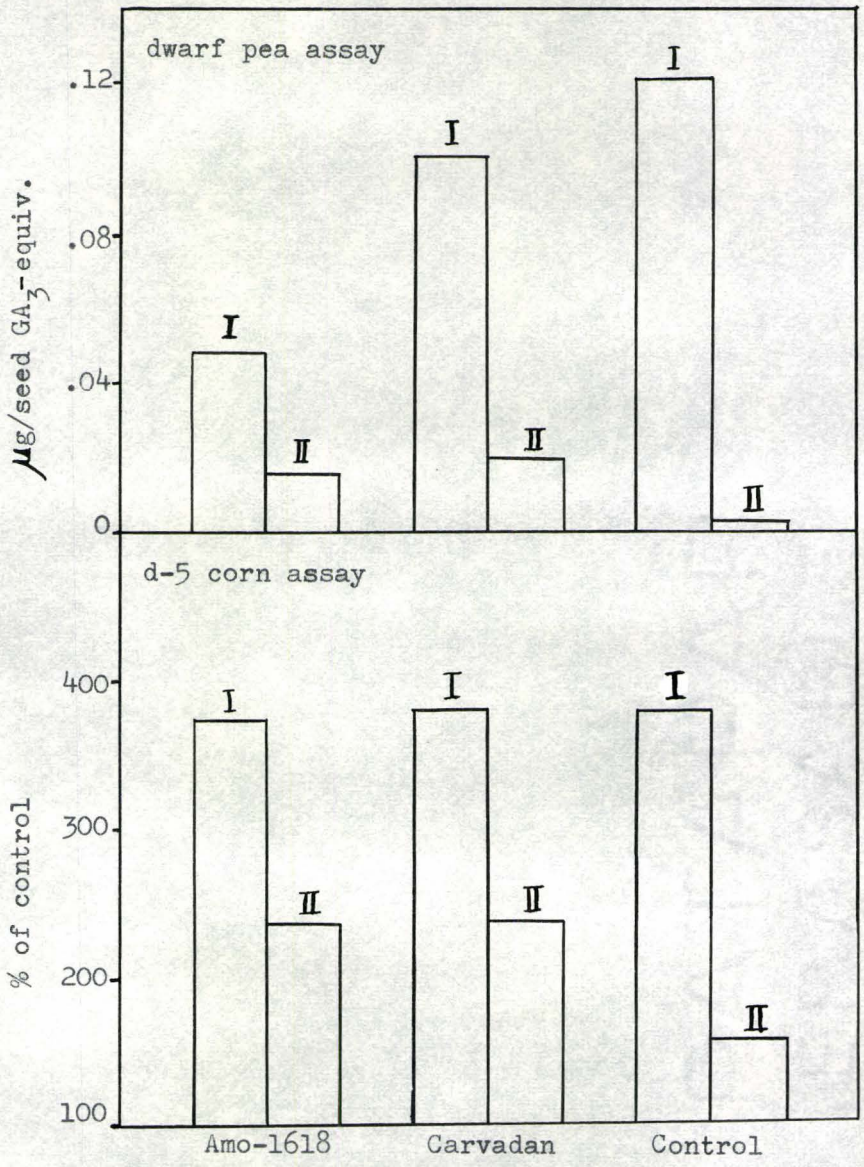


Figure 18. Comparison of growth response in dwarf pea and d-5 corn bio-assay induced by fraction I and fraction II of immature seeds (11 d. after anthesis).

The dwarf pea test gave a value of only 0.016-0.02 µg per seed. This is good evidence that fraction II does contain GA_5, to which d-5 corn ia much more sensitive than dwarf pea. No comparisons can be made concerning fraction I since the d-5 corn was saturated in all three cases.

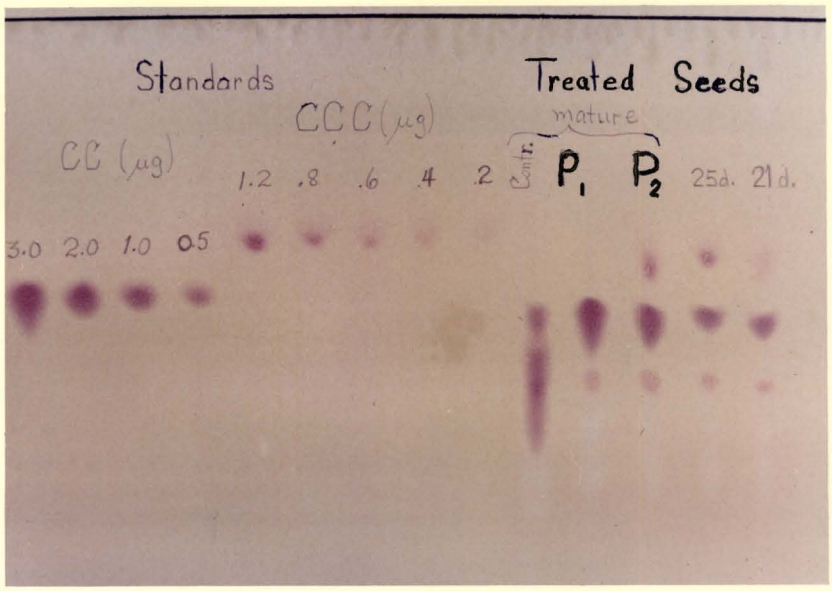
3. Determination of growth retardants in seed extracts

(i) Progeny of CCC-treated plants. TLC on cellulose (ch.II,p.15) of methanol extracts from immature and mature seeds of plants treated with CCC gave the results shown in plate 5. Plate 5 (b) shows TLC of further dilutions of the extracts from mature seeds, since the first chromatogram was over-loaded.

After spraying with Dragendorff's reagent, three spots are visible in extracts of seeds from treated plants. Two of these were identified. A dark pink spot at R_f 0.60 co-chromatographed with, and had the same colour as, CCC; a purple spot at R_f 0.47 appeared to be choline chloride. A pink spot at R_f 0.37 visible in extracts from all bean seeds was not identified. The intensity of the colour of the spot, hence the concentration in the seed, increased as the seeds matured. This substance and choline chloride were present in all seeds, whether from control or from treated plants.

Semi-quantitative estimates of the amounts of CCC and CC present in the seeds, were made by comparison with the standard series applied to the plate. The results are shown in fig.19. Seeds of P_1 began to accumulate the growth retardant approximately 10-15 days after anthesis. These seeds, from plants treated with a total of 200 mg CCC, accumulated 110 µg per seed at maturity. The 68% inhibition of stem growth observed in this progeny (ch.III p.30) was equivalent to that produced by

a.



b.

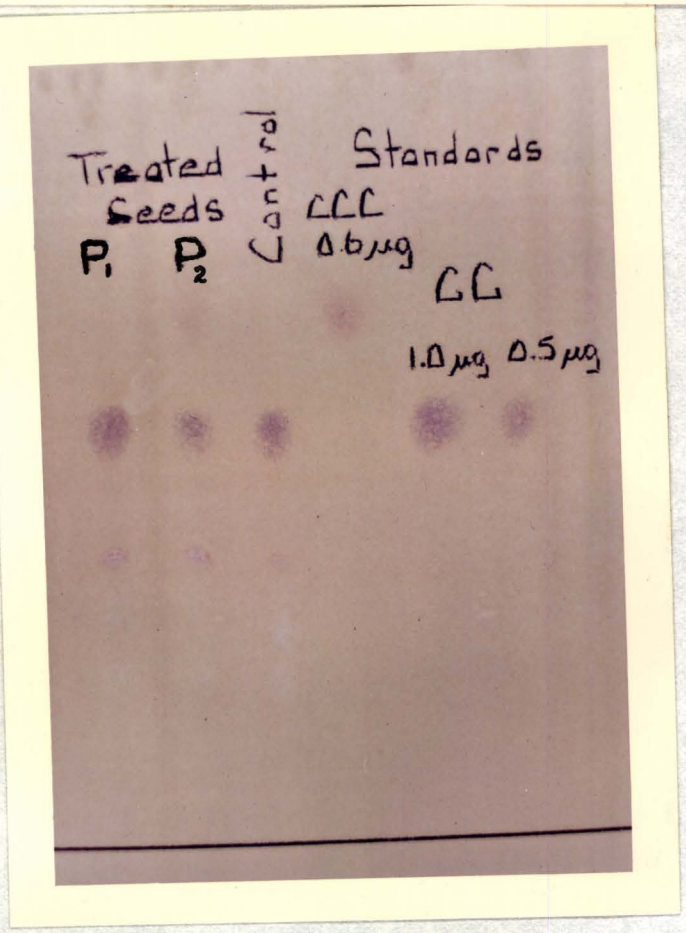


Plate 5. Thin-layer chromatograms of extracts from progeny of CCC-treated plants. "b" shows chromatography of a 1/3 dilution from "a" of samples P_1 and P_2.

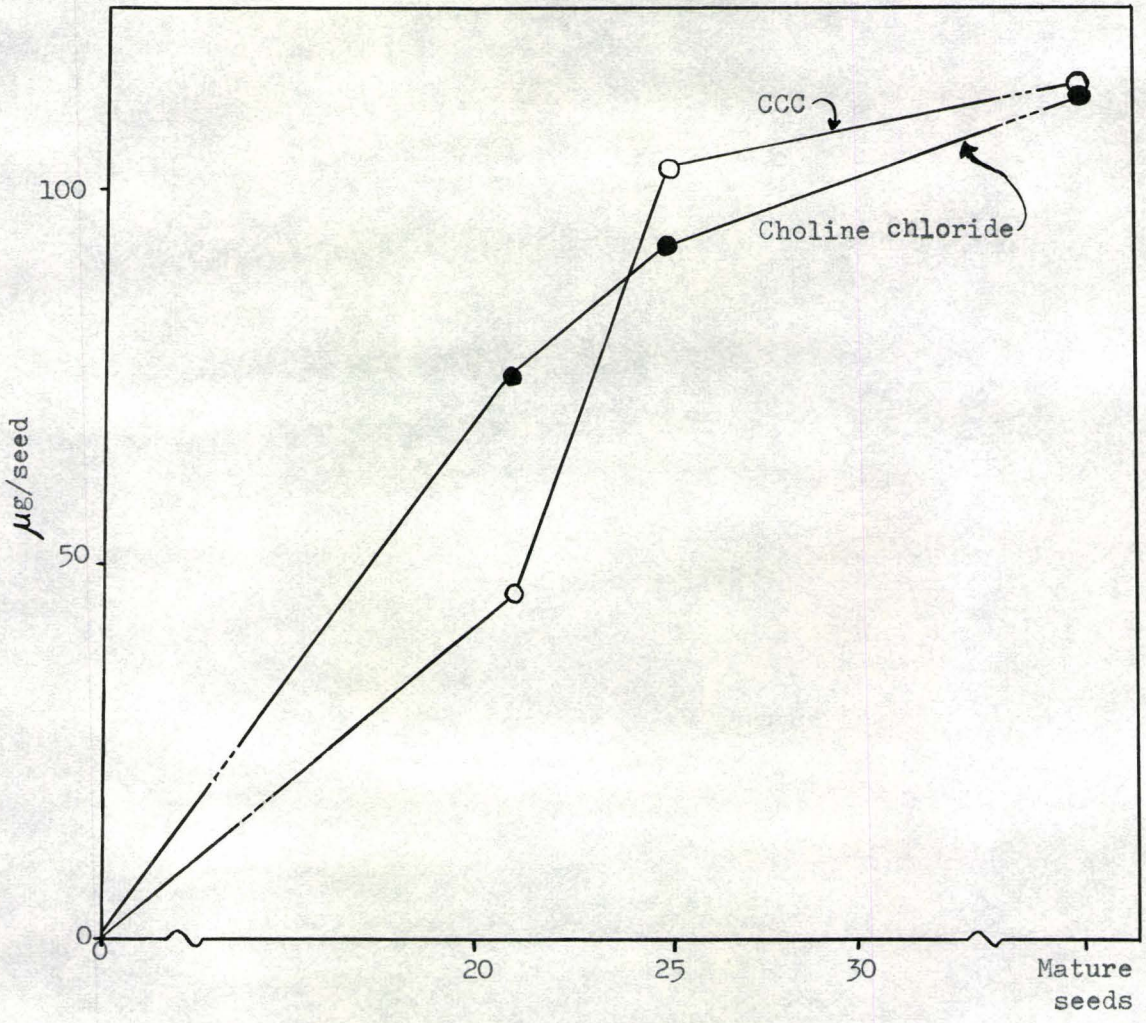


Figure 19. Change in CCC and choline chloride content of P_1 progeny from plants treated with CCC (200 mg).

16 mg CCC applied via the roots (by interpolation, figure 3). Thus, there is sufficient CCC present in the seeds, allowing for incomplete uptake in application via the roots, to account for the inhibition observed in the growth of the progeny. Mature P_2 progeny seeds contained no detectable amounts of CCC, and no inhibition of growth was observed when these seeds were grown (ch.III p.30).

The CC content of P_1 seeds increased as the seeds matured, but remained considerably less than that of control or P_2 seeds. P_1 seeds contained 113 µg/seed CC at maturity, while P_2 control seeds contained 225 µg/seed. These estimates are, of course, semi-quantitative since they are interpolations of intensity of a colour in a standard series.

(ii) Progeny of Amo-1618-and Carvadan-treated plants. Extracts from seeds of plants treated with Amo-1618 or Carvadan showed no pink spot comparable to those of the standards after TLC was carried out as described in ch.II,p.15. However it was discovered that eluates of band 1 from Silica gel H plates (ch.II,p.12), which were run for separation of the GA's in these extracts, contained large amounts of the growth retardant used in treating the parent plants. In the solvent system used for GA separation, (di-isopropyl ether/acetic acid, 95:5), Amo-1618 and Carvadan remain in the origin. This part of the chromatogram was eluted, dried, redissolved in 85% methanol, and run on cellulose plates in the butanol/acetic acid/water (4:1:1.8) system used for growth retardant separation. Upon spraying with Dragendorff's reagent, a bright pink spot appeared at R_f 0.9-0.95, for both Amo-1618 and Carvadan. The proximity to the solvent front, which is usually marked by a thick wavy greenish-yellow line, made it impossible to compare intensities

of the colour of these spots, but the pink colour gave enough contrast to make the spots visible. Thus,although the presence of Amo-1618 and Carvadan in P_1 and P_2 seeds was demonstrated, no quantitative estimates were made. This will be possible when a more appropriate solvent system is used. At the time of writing the use of aluminum oxide plates with butanol/ethanol/water as solvent system was a promising method.

Extracts from progeny of Amo-1618-or Carvadan-treated plants, made and chromatographed as described for CCC, showed the same purple choline chloride spot, and the pink spot at R_f .37. There was considerably less choline chloride in seeds from the treated plants.

CHAPTER V

Discussion and Conclusions

In this chapter the results of the experiments carried out will be discussed with respect to other available data. This will include consideration of where they fit in with present concepts of the mode of action of growth retardants, and where they point to further problems for research.

1. Effectiveness of Growth Retardants.

One purpose of this study was to set up on a more rigorous, quantitative basis the Phaseolus vulgaris system for the study of the action of growth retardants in plants, and their relation to GA. None of the previous work concerning the effect of different types of growth retardants on this plant has included a quantitative comparison of their relative effectiveness, although it is realized that a great variation in effectiveness exists. This applies not only among types of growth retardant, but also among analogues of one type, as several studies have shown (Cathey, 1965; Zeevaart and Osborne, 1965).

As studies of the mode of action in plants proceed to the molecular level, it will be necessary to have available dosage-response curves comparing the growth retardant types with respect to the effect of changes in concentration. The differences in rate of change of these curves are related to enzyme kinetics in the affected

systems. Lockhart (1962) carried out this type of experiment on the response to GA of plants treated with Phosfon-D and CCC, but it has not previously been done with growth retardant concentrations.

Of the four growth retardants studied here, Phosfon-D showed the greatest rate of change (fig.3), followed by B-995, Amo-1618, and CCC. The difference observed between B-995 and Amo-1618 is probably not significant, but certainly Phosfon-D showed the steepest slope, and CCC the least. These results are further discussed on p.55,56.

In interpreting these results, the dose required to give a response must also be considered. Amo-1618 and Phosfon-D are effective at relatively low concentrations, B-995 and CCC at higher concentrations (fig.3). The effectiveness of the four compounds varies over 3 orders of magnitude. The interpretation of these results depends on data concerning uptake, transport, and especially biochemical knowledge of the enzyme system for GA production which is probably being affected by the growth retardants. The results obtained in this study are necessary, but not sufficient, to determine the mode of action of growth retardants.

2. Effect of GA_3 on Retarded Plants

Gibberellin reversed the inhibition of stem elongation caused by all four of the growth retardants tested. Very small amounts of GA were required for reversal, compared to the amounts of growth retardant used in effecting the retardation. 0.1 μg GA_3 was sufficient to overcome almost completely any effect of the growth retardant, so that plants treated with growth retardant + GA could not be distinguished from control plants treated with the same amount of GA. In comparison,

in the case of Amo-1618, where the lowest concentration of growth retardant was used, 110 µg per plant was applied via the roots. This is 1000X the amount of GA_3 applied. Plants treated with B-995 received 8000 µg via the roots. These observations conflict with those of Downs and Cathey (1960) who reported that in the presence of Amo-1618 no increase in height over the normal height could be induced with GA.

At the lower concentrations of GA, the degree of reversal caused by the GA was proportional to the degree of inhibition in the plant, not to the amount of growth retardant. At higher levels of GA, growth was approaching a saturation level where further stem elongation was not possible (fig.4). These results concerning reversal of inhibition by GA agree with those of Lockhart (1962) who measured cms. of growth from the second to the sixth day after treatment. No quantitative comparisons can be made since dosages of the retardants used, Phosfon-D and CCC, and methods of application of the chemicals were different. The effect of means of application on the response produced was pointed out by Preston (1961). Paste applications of Phosfon-D and Amo-1619 gave approximately equal responses, but soil applications of Amo-1619 were much more effective than soil applications of Phosfon-D.

The experiments concerning time of application of GA to retarded plants show some effect of the growth retardants in decreasing the response to GA in older plants. Phosfon-D treated plants showed the greatest difference between response to GA at day 0 and day 10 (fig.10), and were more sensitive to GA treatment on day 0 than plants treated with any of the other three growth retardants (fig.7; figs.6,8,9). CCC-treated plants were least sensitive to GA treatment. There is

obviously a correlation here between sensitivity of the untreated plants to a particular growth retardant, as revealed in the steepness of the dosage-response curve (see ch.V,p.54), and the sensitivity of the treated plants to GA. The minimum dose of growth retardant required to give a response does not appear to be related to the sensitivity to GA.

3. The Growth of Progeny from Retarded Plants

(i) Growth of seeds. Growth retardants did appear to affect the growth of seeds on treated plants, but the effect was not a permanent one. Although the increase in fresh weight and dry weight was delayed by Amo-1618, Carvadan, or CCC, the final weights were not significantly different from those of seeds of control plants. This delaying effect of growth retardants may be brought about through the same mechanism as the inhibition of stem elongation. In Pharbitis nil, the number of days maximal growth rate is delayed is used as a measure of the effectiveness of a growth retardant (Zeevaart, 1964).

(ii) Growth of progeny plants--the transfer effect. Preston (1961) reported that treatments of P.vulgaris var. Black Valentine with Amo-1618, and with four analogues of the compound, resulted in transfer of dwarfing into a succeeding generation through the seeds. Peduncle treatments of Amo-1618 to Black Valentine beans resulted in dwarfing of the progeny, indicating parent plants need not be dwarfed to transfer the response. Dual treatments of Amo-1618 and GA resulted in only the Amo-1618 response being manifested in the progeny. This was the extent of knowledge concerning the transfer phenomenon. The nature of the dwarfing compound or factor which was passed into the seed was not determined, nor were other types of compounds tested for this effect.

Thus experiments were undertaken to further understanding of this phenomenon. The results concerning growth of P_1, P_2, and P_3 progeny plants definitely demonstrate that other growth retardants than Amo-1618 and analogues can cause a dwarfing in the progeny of treated plants. Plants treated with CCC showed the response in one generation following treatment; later generations exhibited normal growth.

Results with Carvadan-treated plants and progeny clearly show that the dwarfing response can be passed on for more than one generation. Both P_1 and P_2 progeny plants were dwarfed. GA applied to the P_1 generation of Carvadan-treated plants, although it caused stem elongation and allowed good fruit set, did not prevent transfer of dwarfing to the P_2. This agrees with Preston's (1961) observations from dual treatments with Amo-1618 and GA.

The dwarfing response in the progeny was not due to toxicity of the chemicals. This was shown by application of GA to the P_1 progeny of Carvadan-treated plants. GA reversed the inhibition, resulting in plants of normal height and taller.

By the GA assay methods used in these experiments it could not be determined directly whether the transfer of dwarfing was related to a decreased GA content in seeds from treated plants. The amount of GA present in the seeds at the time of maturity was not detectable in any of the progeny, including those of control plants. More sensitive assay methods, different extraction procedures, and the use of larger samples may make it possible to determine the amounts of GA present. However it is unlikely that decreased GA content in the mature seeds is the cause of the transfer of dwarfing to the progeny, since applica-

tion of GA to treated plants (Preston, 1961) or to dwarfed P_1 progeny did not prevent transfer of the dwarfing effect.

More direct evidence that decreased GA content is not the cause is the detection of the presence of the growth retardants themselves in the seeds. CCC was shown to be present in amounts sufficient to cause the observed response. Amo-1618 and Carvadan were also identified, although the amounts present could not be determined. Thus it seems clear that the transfer of the dwarfing response is actually a direct transfer of the growth retardants themselves, and not of some other "dwarfing factor", or more indirect effect. GA can reverse the dwarfing of the progeny because exactly the same mechanism is operating as in plants treated directly with the growth retardants. As a conclusive check, experiments should be carried out simultaneously with P.vulgaris and P.multiflorus, in which transfer does not occur (Preston,1961), comparing growth retardant distribution in the plant and fruit, GA content, seed growth, and growth retardant accumulation in the seed.

Further chemical work should be done on the Amo-1618 and Carvadan accumulated in seeds, in order to determine how these substances are bound. This information could conceivably throw some light on the mode of action of growth retardants at the molecular level. Other retardants should also be studied with respect to the transfer phenomenon.

More difficult to interpret is the effect of growth retardants on GA content of the immature seeds. Their effect was roughly proportional to their effectiveness in retardation of stem growth. GA-level was greatly reduced at the time when the content reached a maximum, but the differences disappeared as the amounts decreased with age. No

effect could be seen on final weight of the seeds, either fresh or dry. Perhaps the decreased GA_1-content is related to the delay observed in seed growth (see ch.V, p.56). The GA_5-content of the seeds from treated and control plants is difficult to interpret, although retardants do have some effect here, apparently causing an earlier peak. At the intervals at which samples were taken, however, it is possible that other peaks were missed.

For determinations of both GA_1 and GA_5-like material it would be useful to make extracts from many more seeds, at shorter intervals, and beginning sooner after anthesis, in order to follow more closely the GA changes. An earlier GA_5 peak may be found, as Jones observed with P.multiflorus (1964). GA_5 content should be tested at each stage on d-5 corn, which is more sensitive to this GA. This dwarf corn mutant is not at present available in the large quantities necessary to obtain conclusive results.

APPENDIX

One-Half Strength Modified Hoagland Solution

(Earhart lab.)

Nutrient		Grams per litre stock solution
Solution A		
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	295.0
Sequestrene	NaFe, 13%	38.44
Solution B		
Potassium Phosphate	KH_2PO_4	34.25
Potassium nitrate	KNO_3	126.65
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	126.65
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	0.056
Manganous sulphate	$MnSO_4 \cdot H_2O$	0.391
Copper sulphate	$CuSO_4 \cdot 5H_2O$	0.021
Boric acid	H_3BO_3	0.725
Molybdic acid	$MoO_3 \cdot 2H_2O$	0.005

1. Both stock solutions "A" and "B" are to be used at 1:500.

2. Stock solutions "A" and "B" should not be mixed together when undiluted.

Dragendorff's Reagent[1]

To make Stock Solution:

2.6 g Bismuth subnitrate ($BiONO_3$)

7.0 g NaI

25 ml Acetic acid

Reflux, cool and filter.

20 ml filtrate
80 ml Acetic acid
} STOCK SOLUTION

Dilution for Use:

10 ml stock solution

50 ml Acetic acid

120 ml Ethyl acetate

With constant shaking add 10 ml H_2O dropwise.

[1]Thies, H., and F. W. Reuther: Ein Reagens zum Nachweis von Alkaloiden auf Papierchromatogrammen. Naturw. 41, 230-231 (1954).

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