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Experiment With the Plant Growth Hormone Gibberellin

Jeanette A. Baker
Southern Illinois University Carbondale

Botany 492

Jeanette Baker

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Introduction:

The plant growth hormone gibberellin causes cells in the stem internodes of plants to elongate. This has been demonstrated in both normal and dwarf plants (Scott, 1975).Cabbages have been induced to grow to two meters tall by the correct application of a gibberellin (Stern, 1985). Response to a treatmemt with gibberellin has been shown to be quite rapid (Carr, 1972).

The effect of gibberellin on plant growth was first described in Japan in 1809 as a desease of rice plants called "bakanae" or foolish seedling. This desease caused affected seedlings to beccome thin, pale green, and much taller than uninfected plants.

In 1895 Hori identified the cause of this problem to be an imperfect form of the fungus Gibberella fujikuroi. In 1926 Kurosawa grew G. fujikuroi and obtained a cell free extract from the growth medium. He treated rice and maize seedlings with this extract and obtained the same effect as rice growers had observed and called "bakanae".

Since that time, 24 gibberellins have been isolated from the fungus G. fujikuroi. Among these is

gibberellic acid (GA_7). In 1958 MacMillan and Suter isolated GA_1 from immature seeds of Phaseolus coccineus. This was the first isolation of a gibberellin from a higher plant. Now 43 of the known 57 gibberellins have been found in higher plants. Also many gibberellins have been synthesized. The first to be synthesized was GA_3. This was done by Corey et al. in 1978 (MacMillan, 19). Work is still continuing on the synthesis of gibberellins.

Purpose of this experiment:

The purpose of this experiment was to test the effects from treating ga-1 mutants of Arabidopsis thaliana with GA , GA ,and GA at differing concentrations and differing time schedules with particular interest directed toward finding how late treatments can be delayed and how small amounts of Ga can be given without decreasing height and seed production from that which is obtained from four weekly treatments of GA at 10 molar concentration with the first treatment at two weeks from the day the seeds were planted. However, at four weeks into this experiment the circulating fan on the growth chamber containing the plants stopped functioning. This allowed the chamber

to heat up to about 90 degrees F. After this the plants began to mature ealier than is usual and therefore there was not a measureable seed production so I was not able to consider effects on seed production at this time. Also these plants did not attain the final height that is usually found with these types of treatments, but comparisons can still be made as to the differences in heights since all the plants were subjected to the same heat. It would of coarse have been best to begin the experiment over, but time was not available to do this.

Design of experment:

This experiment was set up to be analyzed as a Model I three-way ANOVA. The dependent variable to be measured was plant height after six weeks of growth from the date the seeds were planted (seed weight would also have been measured if this had been possible). The alpha level chosen was 0.05. The analysis was done by computer using SAS.

Factors were:

GA

with levels of:

GA_3

GA_{4+7}

GA_{20}

Concentrations

with levels of:

10^{-3}

10^{-4}

Treatments

with levels of:

A One microliter at two weeks and each week after for three more weeks

B One microliter at two weeks only

C One microliter at two weeks and at three weeks

D One microliter at three weeks only

Sample size was 10 plants for each group making a total of 240 plants for the experiment. I did a pilot study earlier which indicated that due to the variance of associated with these plants, a sample size of 30 would have been desirable. However, this was not possible due to space limitations and the fact that this experiment was designed to fit into a larger experiment which is ongoing and has a sample size of 10.

Methods:

Growth medium:

Hoagland's solution was prepared and pH adjusted with sodium hydroxide to pH 5.6. This was solidified with .5% agar. 15 ml of this was added to each 20 x 200 mm culture tube which was capped with a plastic cap. These were sterilized in an autoclave for 20 minutes.

Procedure for starting seeds:

Seeds of ga-1 mutant of Arabidopsis thaliana were soaked for 10 minutes in 1/4 strength Clorox liquid bleach with two or three drops of dishwashing liquid added as wetting agent. Then these were rinsed three times in sterile demineralized water. Next the seeds were soaked in GA_3 10^{-3} for one hour. This is necessary for germination of these ga-1 mutant seeds. These were again rinsed three times in sterile demineralized water. The seeds were then planted with a sterile pipette onto a petri dish containing 20 ml of Hoagland's solution solidified with 2% agar.

Culture:

Plants were grown in a Sherer Gillett growth chamber manufactured in Marshall, Michigan.

Treatment:

Treatments were administered with an Eppendorf digital pipette 4710 micropipeter. GA_{20} and GA_{4+7} were not soluble at 10^{-3} molar concentration. Therefore, in order to administer a one microliter 10^{-3} treatment, the plants were given five microliters of the appropriate GA at 10^{-4} one day and five microliters more the next day. This was done in two five microliter treatments instead of one ten microliter treatment because of the difficulty in keeping a ten microliter drop from falling off the plants. All other treatments were one microliter.

Results:

The ANOVA showed a significant difference in the means of the plants due to the different gibberellins and due to the different concentrations and due to the different treatments. There was also a significant interaction between concentration and treatment. This was the only interaction found. Since there were significant differences due to all three factors, the Duncan means comparison test was performed to find where these differences were located.

Among the gibberellins, GA_3 and GA_{4+7} were found to act with no significant differences. However, GA_{20} gave means that were signifcantly lower than the others.

Between the two different concentrations, there was a significant difference with the 10^{-3} molar giving significantly higher means than the 10^{-4} molar concentration.

Among the treatments, there were found to be significant differences for each of the treatments. The highest mean was obtained from treating the plants with one microliter at two weeks and one microliter each week after for three more weeks. The next highest mean was obtained from treating with one microliter at only the third week. The next highest mean was from treating with one microliter at two and at three weeks. The lowest mean was from treating with one microliter at two weeks only.

Discussion:

The results from the gibberellins and from the concentrations are hardly surprising since similar results have been obtained in other experiments. The concentration-treatment interaction should be

investigated in futher experiments. The results of the comparisons of the treatment means were very interesting. It does not seem strange that the treatment with one microliter at week two and each week after for three more weeks gave the highest means since this treatment supplied the plants with more gibberellin than the other treatments. However, the treatment which gave the next highest amount of gibberellin did not give the next highest means. This was the treatment with one microliter at week two and at week three. The means for this treatment were third from the highest being preceded by means for the treatment with one microliter at only the third week. This seems very strange since both of these groups of plants had GA applied at week three, yet the group which had the additional treatment at week two grew less. The group with the lowest mean was the treatment with one microliter at week two only. It is interesting that this group did less well than the plants treated only at three weeks. This certainly should be investigated further in furture experiments.

Summary:

This experiment shows that the time and amount of

treatment as well as the type o do affect the height of the plants. In the future I would like to repeat this experiment since it was adversely affected by the failure of the growth chamber. Also I would like to expand it in the area of treatment times and amounts in order to find the most effective treatment regime for these plants. Also one very important question which I was not able to address with this experiment concerns the most effective time to treat these plants in order to attain seed production. Flowering and seed production in these plants has been shown to be GA dependent (Benzinger, 1983)

Literature cited:

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Introductory Plant Biology, Kingsley R. Stern, Wm. C. Brown Publishers, Dubuque, Iowa , 1985

Plant Growth Substances 1970, Ed. D.J, Carr Springer-Verlag, Berlin, Heidelberg, New York, 1972

The Effects of Gibberellin, Brasinolide, and Other Growth Regulators on a Gibberellin-Deficient Mutant of Arabidopsis thaliana, Elizabeth A.benzinger, Bachelor of Arts, Southern Illinois University, Carbondale, IL, 1983

Key to Graphs:

A = One microliter treatment at two weeks at each week after for three more weeks

B = One microliter treatment at two weeks only

C = One microliter treatment at two weeks and at three weeks

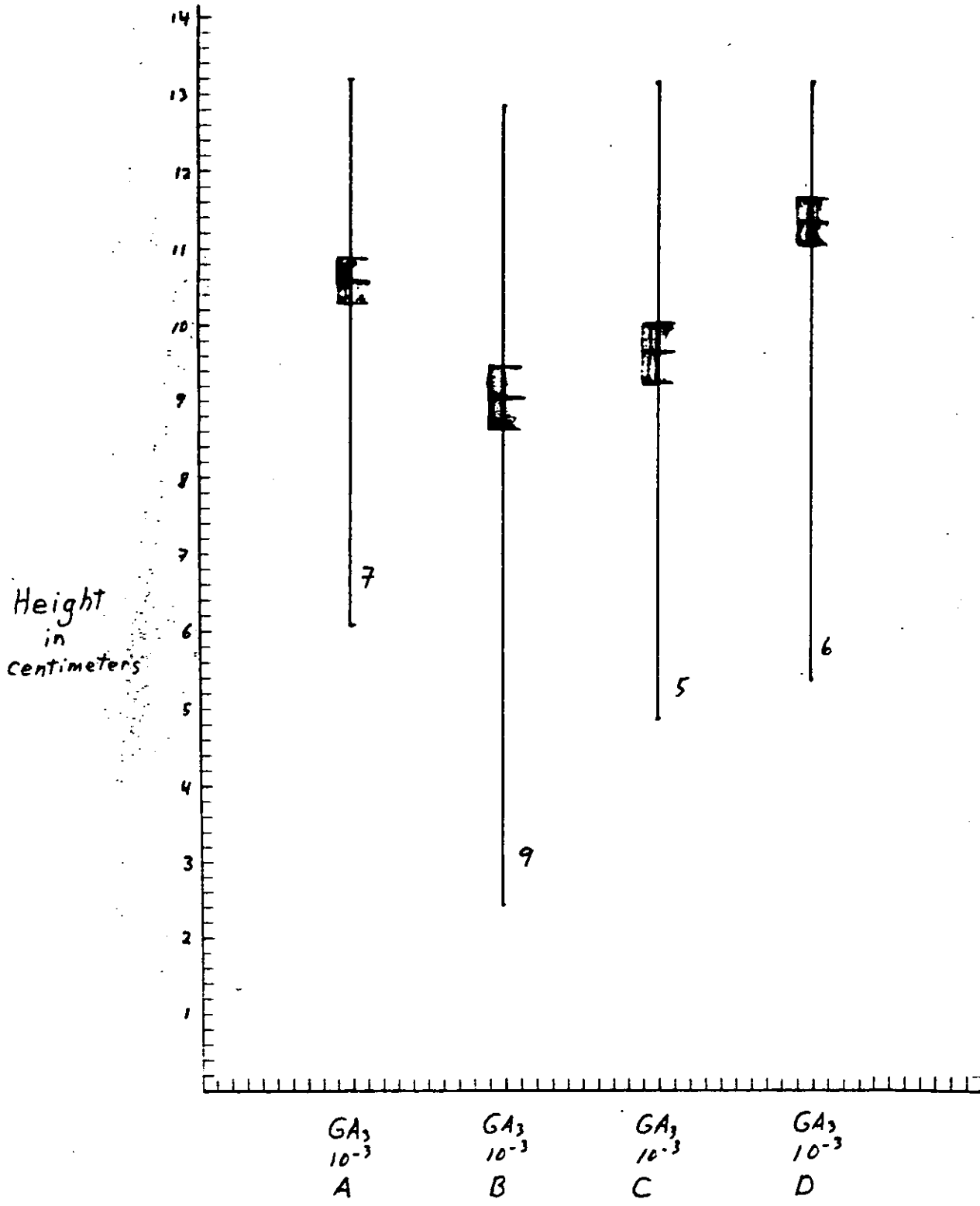
D = One microliter treatment at three weeks only

Verticle line = range

Horizontal line = mean

Shaded area = + or - standard deviation

Small number at bottom of range line = sample size


Height in centimeters
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13
12
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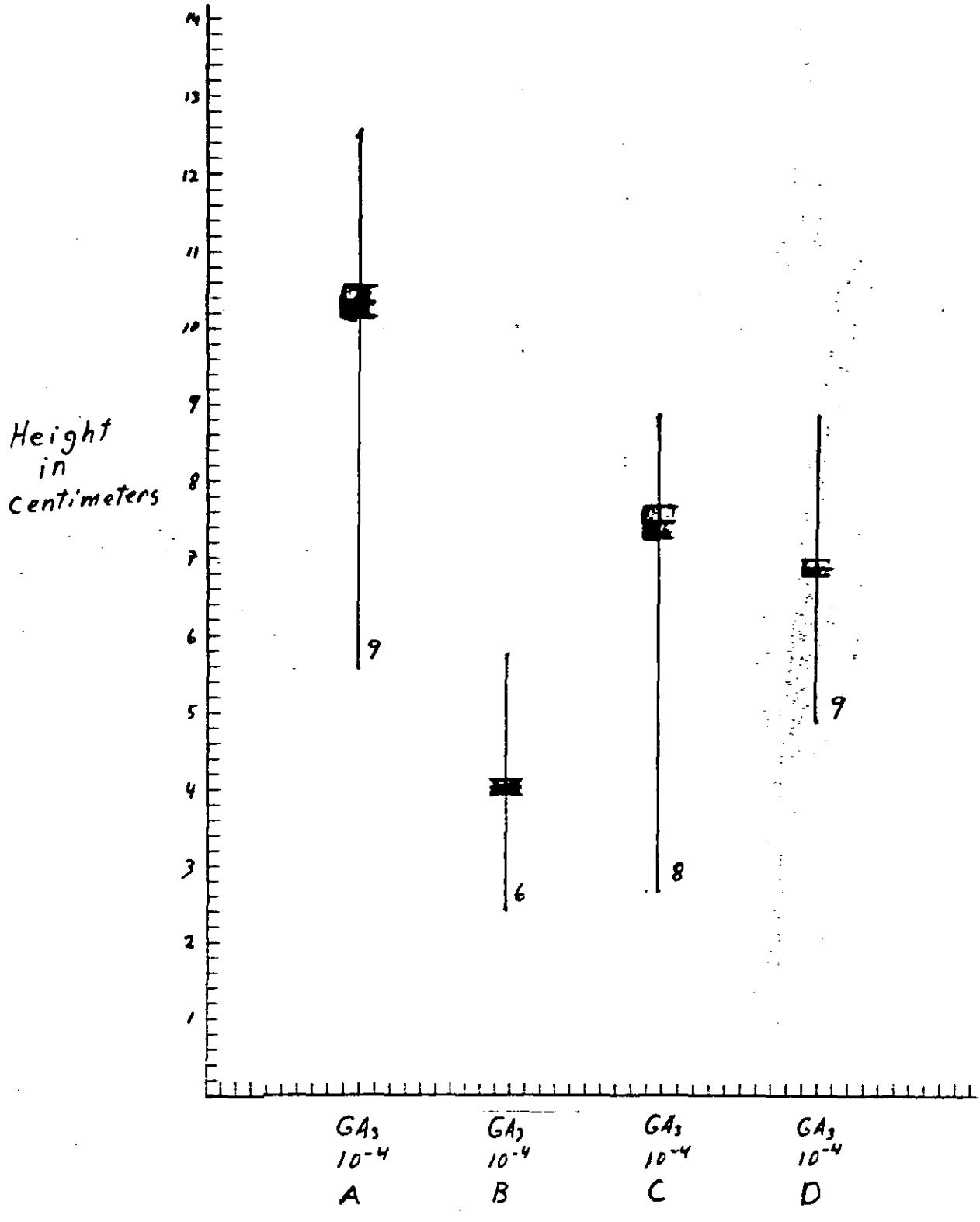
GA3 10-3 A
GA3 10-3 B
GA3 10-3 C
GA3 10-3 D


Height in centimeters
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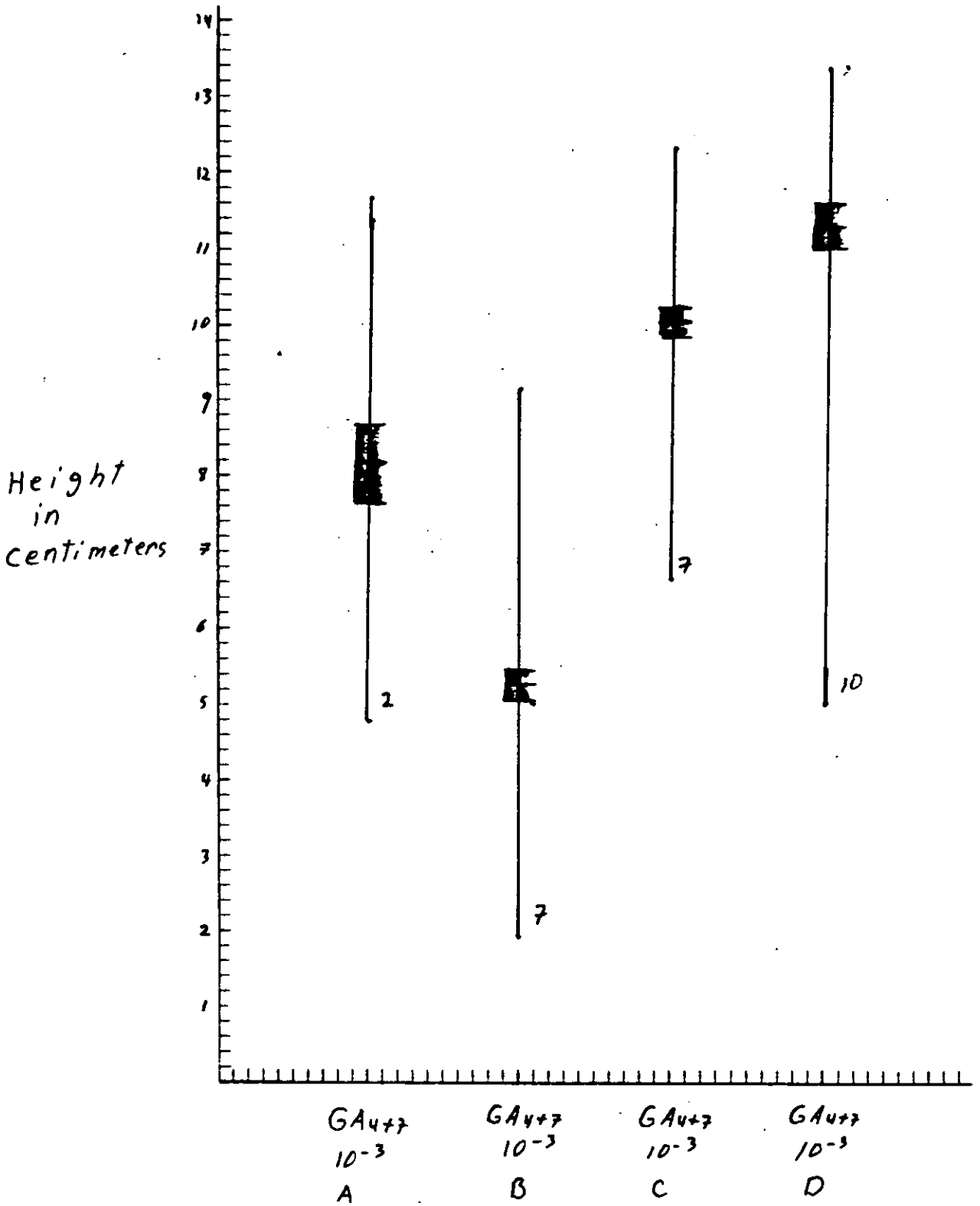
GA3 10-4
C
GA3 10-4
D


Height in centimeters
14
13
12
11
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9
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3
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1
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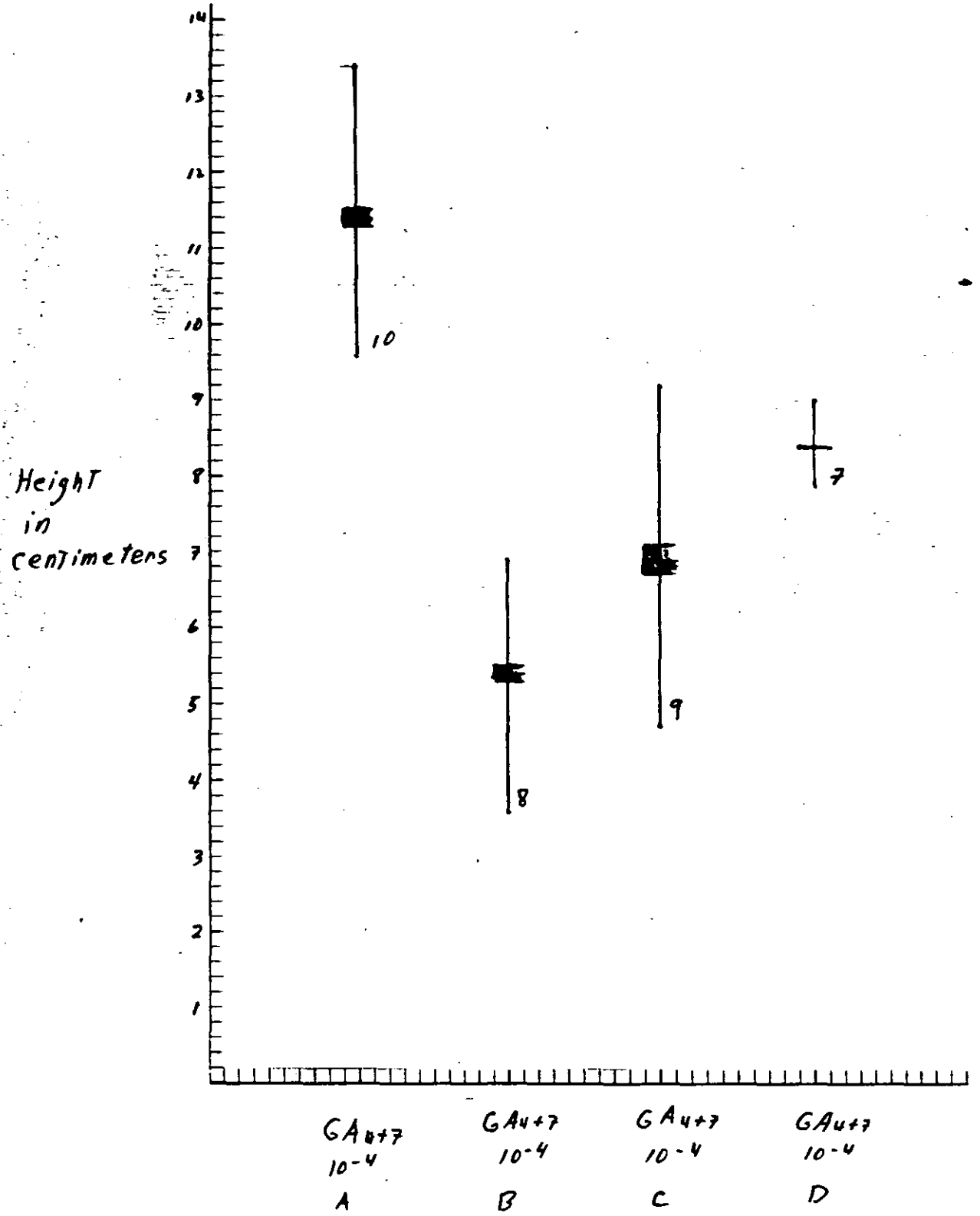
7
10
GAu+7 10-3 A
GAu+7 10-3 B
GAu+7 10-3 C
GAu+7 10-3 D


Height in centimeters
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11
10
9
8
7
6
5
4
3
2
1
10
8
9
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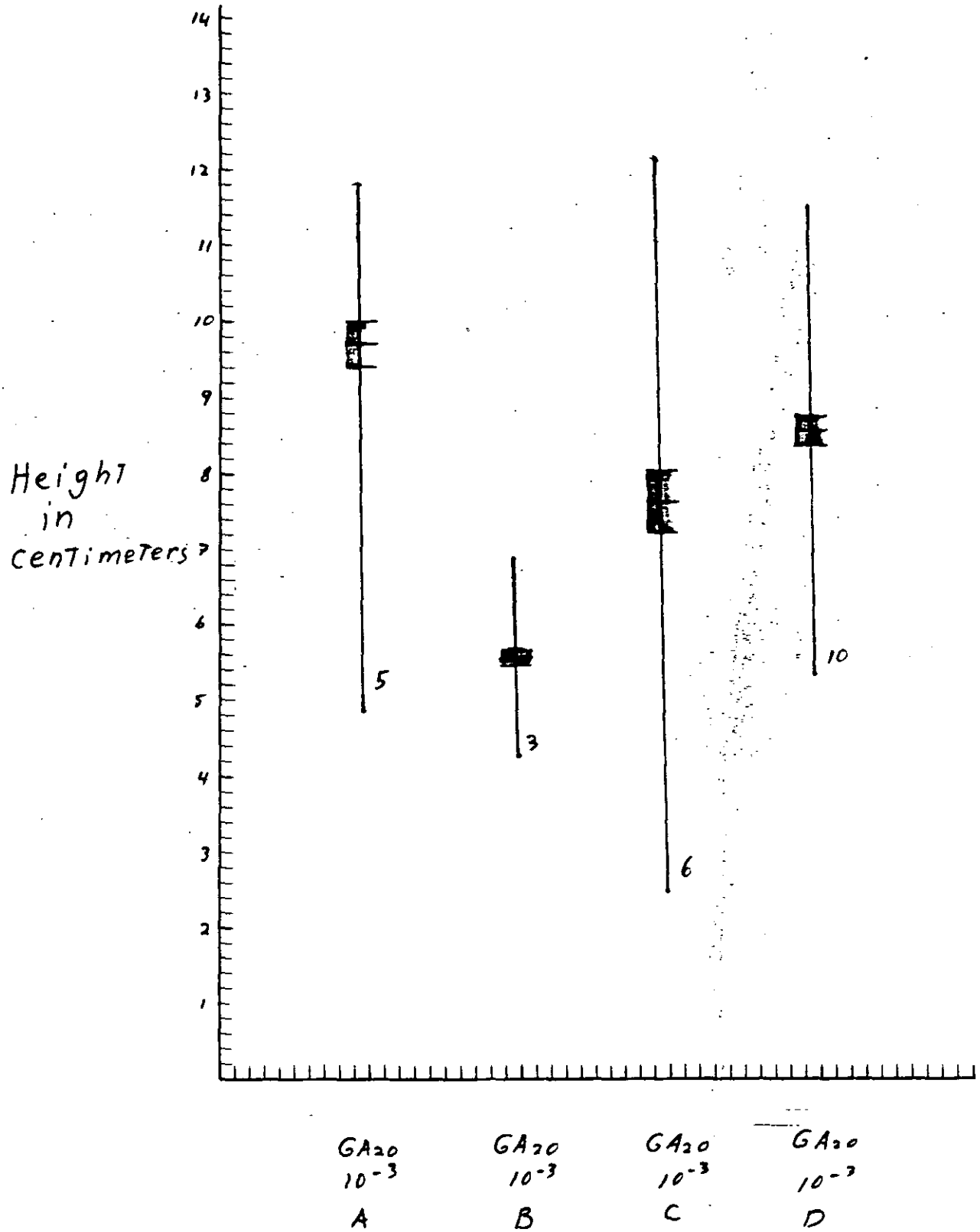
C
GA4+7 10-4
D


Height in centimeters
14
13
12
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8
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6
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4
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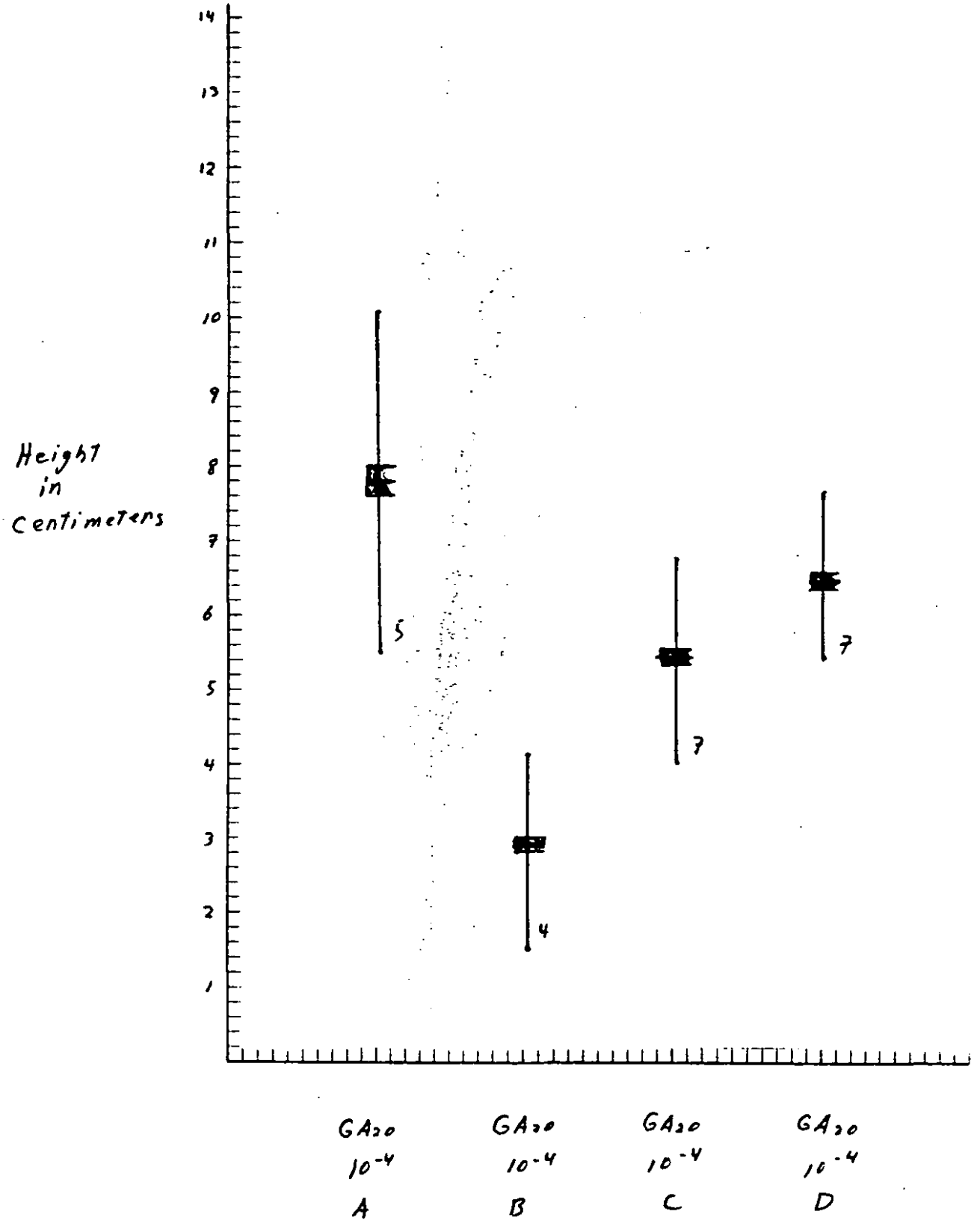
GA20 10-3 D


Height in Centimeters
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8
7
6
5
4
3
2
1
5
4
7
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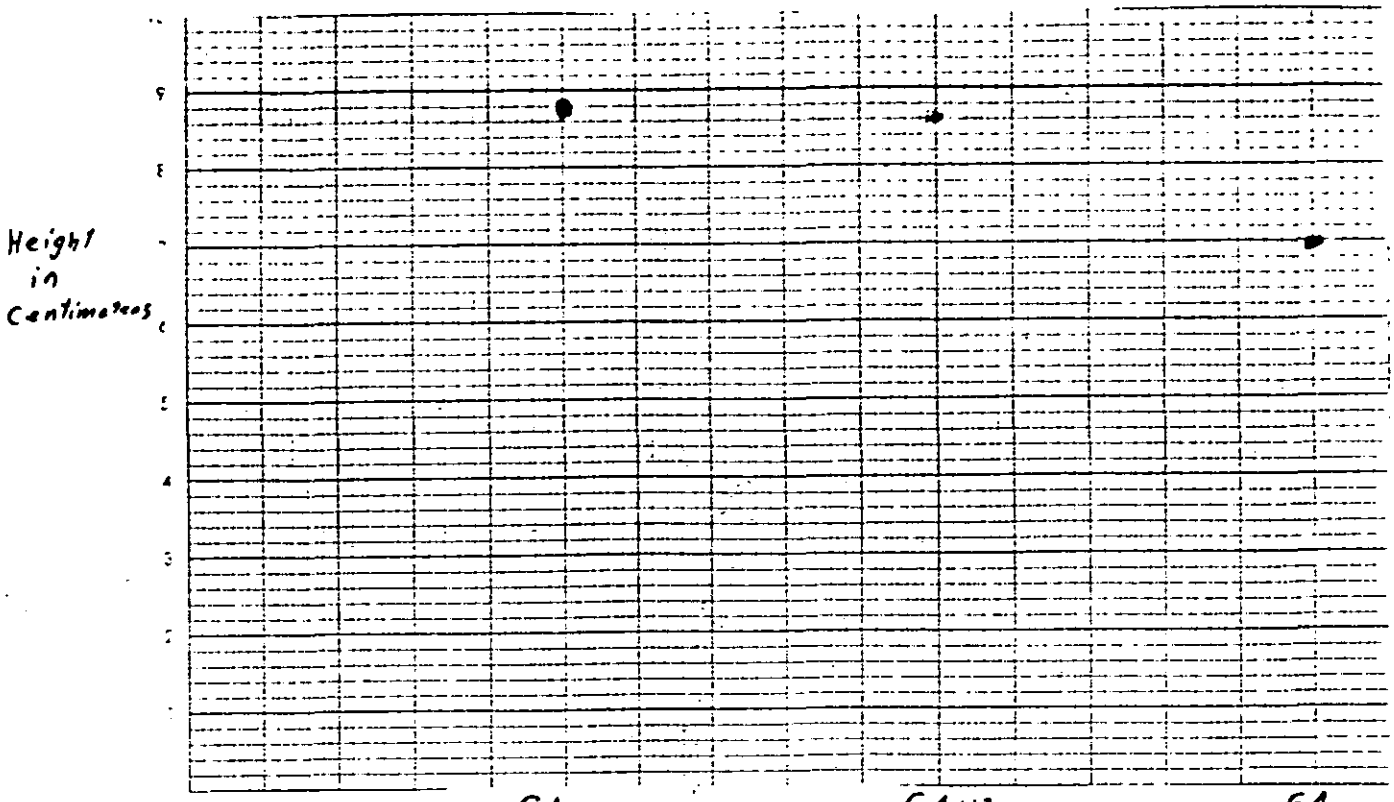
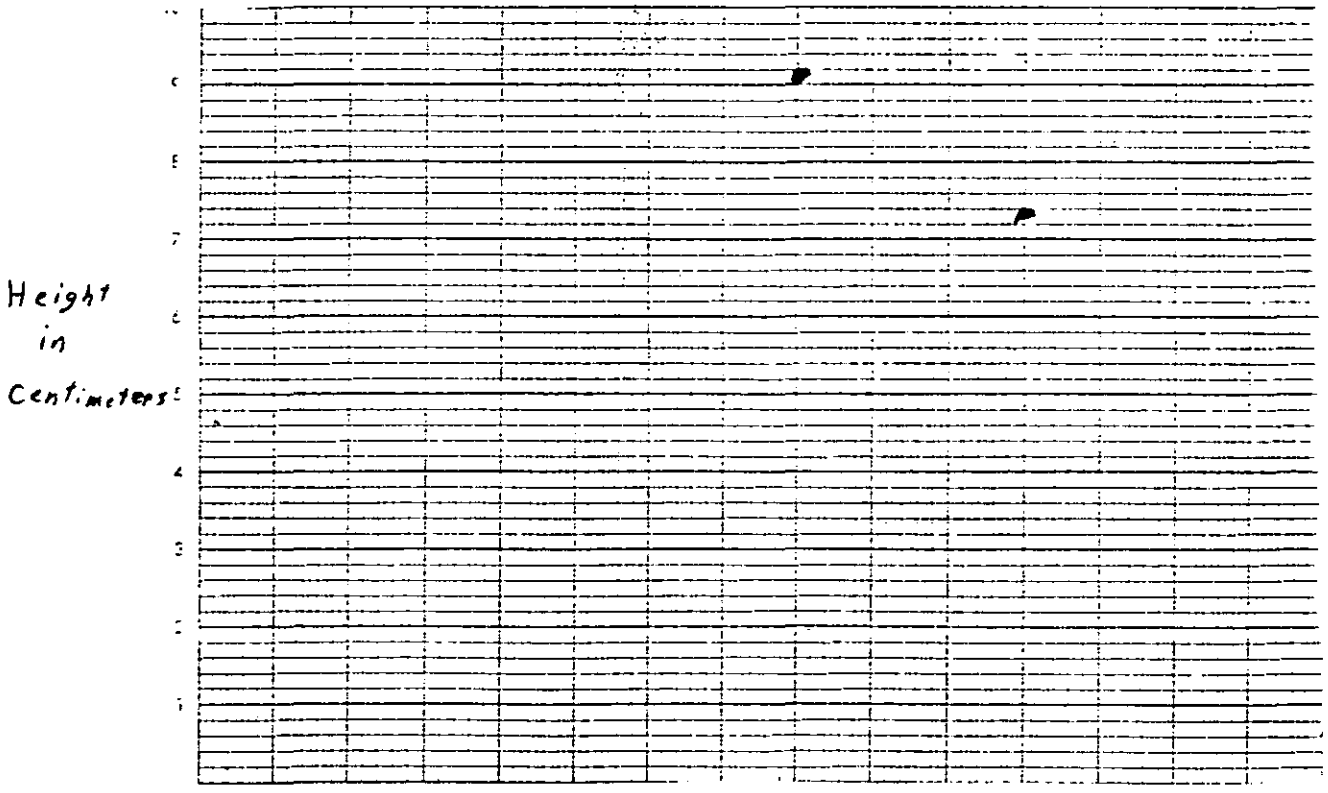
GA20 10-4 A
GA20 10-4 B
GA20 10-4 C
GA20 10-4 D


Height in Centimeters
9
8
7
6
5
4
3
2
GA
Means
GA3
n=59
GA4+7
n=60
GA20
n=47


Height in Centimeters
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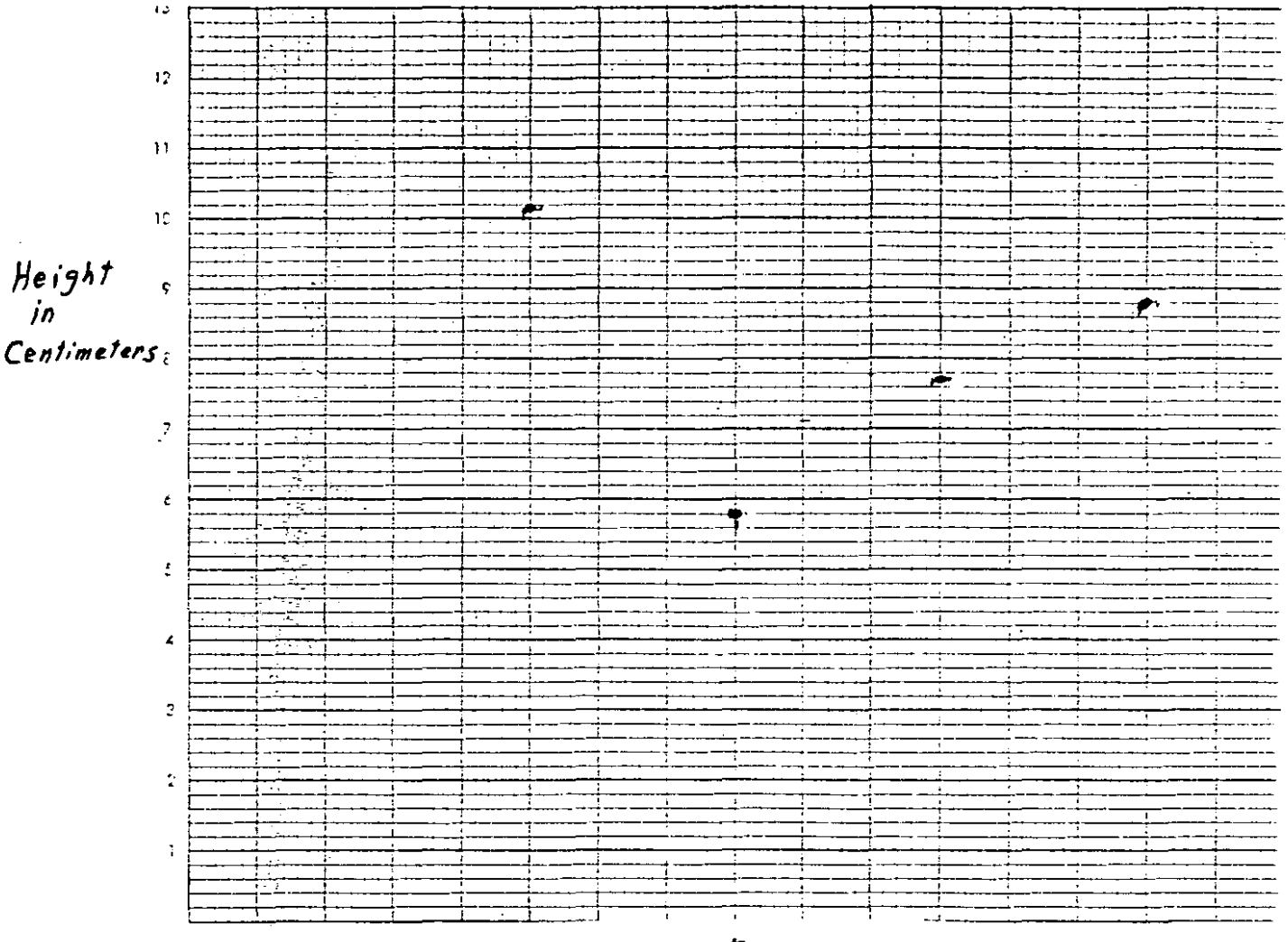
7
6
5
4
3
2
1
Concentration
Means
10-3
n=77
10-4
n=89



Treatment

Means

n = 38 n = 37 n = 42 n = 49

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This is a continuation of the experiment began in the Fall of 1987. In that experiment, GA_3 , GA_{4+7}, and GA_{20} were used in treating the plants. Since it was shown that GA_3 and GA_{4+7} gave plant heights with no significant difference in the means, and that GA_{20} gave heights with significantly lower means, only GA_3 was chosen as the GA for this set of treatments.

Since the 10^{-3} molar concentration gave plant heights with significantly higher means than did the 10^{-4} molar concentration, 10^{-3} was the concentration chosen for this experiment.

For this experiment three different dosage levels were used. Plants were treated with 1 microliter, 5 microliters, and 10 microliters to see if the amount of GA given at the various treatment times would affect the mean height of the plants.

The treatment scheme used in this experiment was as follows:

Treatment A : Plants were given GA_3 at 10^{-3} molar concentration at six weeks from the date the seeds were planted. This was done at the 1, 5, and 10 microliter dosage levels.

Treatment B : Plants were given GA_3 at 10^{-3} molar at 1, 5 and 10 microliters at five weeks from the date the seeds were planted.

Treatment C : Plants were given GA_3 at 10^{-3} molar at 1, 5, and 10 microliters at both week

five and week six.

Treatment D : Plants were given GA_3 10^{-3} molar at 1, 5, and 10 microliters at week two.

Treatment E : Plants were given GA_3 10^{-3} molar at 1, 5, and 10 microliters at weeks 2, 3, 4, and 5.

Treatment F : Plants were given GA_3 10^{-3} molar at 1, 5, and 10 microliters at weeks 2 and 3.

Treatment G : Plants were given GA_3 10^{-3} molar at 1, 5, and 10 microliters at week 3.

General methods and culture conditions were the same as set up in the earlier part of the experiment.

Again the data were analysed using ANOVA to test for differences in means and the Duncan means comparison test was used to find where any observed differences were located.
This time there was not the problem with growth chamber failure causing the plants to suffer from high heat stress. The plants were allowed to grow for 10 weeks to give good seed production so the weights of seeds from the plants were also analysed.

Results : The dose (1, 5, or 10 microliters) made no

significant difference in the mean height of the plants. However, there was a significant difference due to the time

of treatment. Treatment E gave the highest mean and this was significantly higher than that given by any of the other treatments. Treatments D, G, and F gave the next highest group of mean heights. Treatment A gave the lowest mean and this was significantly lower than the other means. However, Treatments B and C gave means which could not be separated totally from all the others. These means were grouped with the means from treatments D, G, and F and were also grouped with the mean of treatment A. The interaction between dose and treatment was significant.

The effect was sort of reversed for the means of the seed weights. In this respect the different treatments produced no significant differences, whereas, the dosage did produce significantly different means in the seed weights. The 1 microliter dose gave the highest mean, and this mean was significantly higher than the five microliter dose with the 5 microliter dose giving the lowest mean. The mean for the 10 microliter dose was grouped with both the 1 and the 5 microliter doses as it was between these two and could not be placed absolutely in with either of them even though likewise it could not be absolutely separated from either of them. The interaction between dose and treatment was also significant.

Discussion :

Since the lowest dose of GA gave the highest mean for the seed weight, it would seem that the amont of GA applied at a treatment time is not directly proportional to the

weight of seed produced. In fact it would almost seem that the opposite were true except that the 10 microliter dose did not give lower means than the 5 microliter dose. Altogether, this is a little puzzling and probably needs further investigation in future experiments especially since there was a significant interaction betweeen dose and treatment.

It is very interesting that the week of treatment and the number of weeks the treatment was given had no effect on the mean weight of the seeds. This seems especially interesting in light of the fact that these ga-1 mutant plants do not produce seed at all if they are not treated with some amount of GA.

Since the dosage made no significant difference in the mean height of the plants it would seem that even 1 microliter of GA 3 at 10 molar provides sufficient GA for elongation of the plant stem. It also seems to show that additional GA of at least up to the 10 microliter dose produces no detrimental effect on the plant height.

Even though the treatment schedule did not affect the seed production, it did influence plant height. Treatment E gave the highest mean. This is also the treatment which gave the plants the greatest number of treatments which started at week two, which is the earliest treatment given. As far as plant height is concerned, it seems that early treatment is important since the second highest mean was the result of treatment D which gave the plants one treatment of GA at

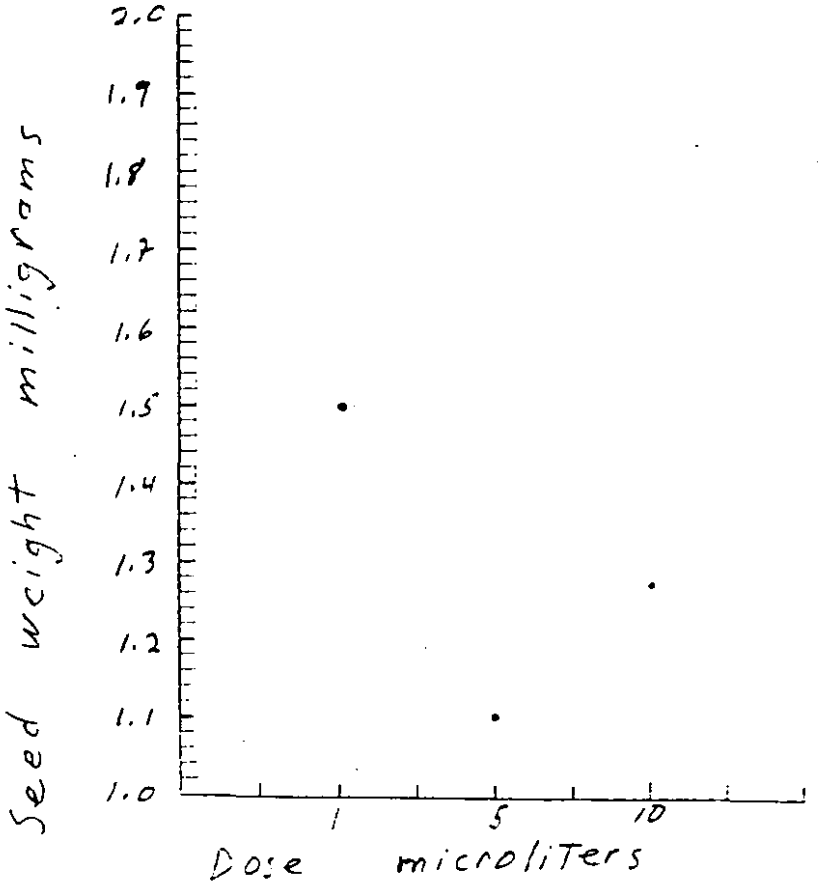
week two only. Again this time as in the earlier part of the experiment, the height mean was greater for plants treated at week three only than for plants treated at week two and week three.

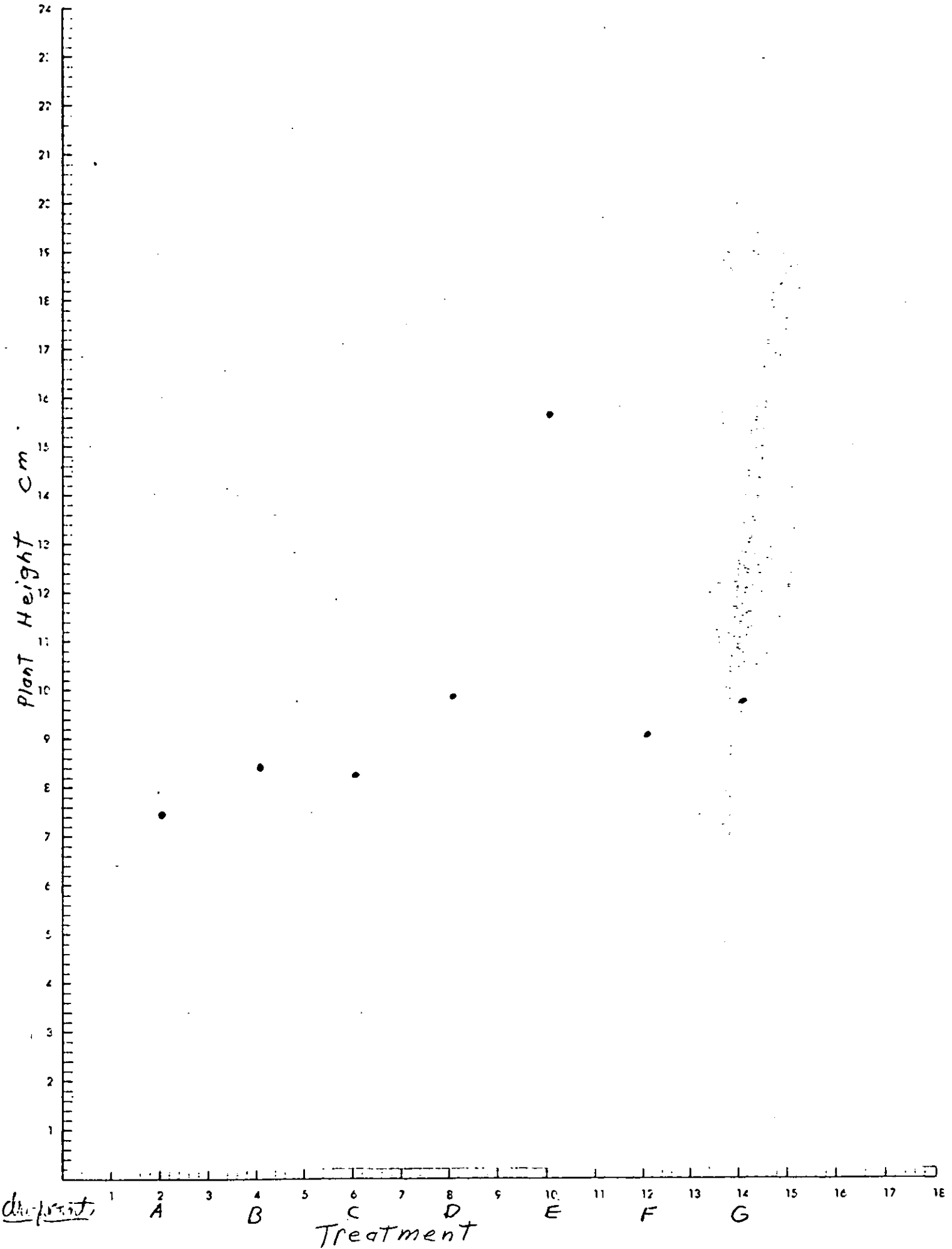
Summary :

In this experiment seed production was not affected by the various treatment schedules but was affected by dosage. The fact that the higher dosages gave lower seed weights shows that more experiments need to be done to further investigate possible causes for this.

The interaction between dosage and treatment for both seed weight and plant height needs further investigation.
The fact that treatment and dosage seem to have opposite effects on plant height than they do on seed weight even though GA is necessary for seed production and for stem elongation in these plants needs further study.

The effect of the three week only treatment on plant height is very interesting. More extensive experimentation in this area might reveal some information as to the ideal time schedule for treatment of these plants.


2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
Seed weight milligrams
1
5
10
Dose microliters


Plant Height cm
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16
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18
19
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22
23
24
1
2
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7
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17
18
A
B
C
D
E
F
G
Treatment