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Summary: The purpose of this report is to acquaint the high school biology teacher with some of the plant growth regulators, the effects they have on plants, and to provide some introductory experiments for use in the high school. A historical background was included so as to make the work more meaningful to those who are unfamiliar with but interested in this particular field. A portion of the report indicates where new ideas for experiments with plant growth regulators may be secured and also a partial list of where plant growth regulators may be purchased.

ADVISER'S APPROVAL Jerry V. Holt

PLANT GROWTH REGULATORS AND THEIR USE IN HIGH SCHOOL BIOLOGY

By

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PLANT GROWTH REGULATORS AND THEIR USE

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INTRODUCTION

Statement of the Problem

The purpose of this report is to acquaint the high school biology teacher with some of the plant growth regulators, the effects they have on plants, and to provide some introductory experiments for use in the high school. Hundreds of books and articles have been published in the last decade pertaining to plant growth substances. This report does not attempt to review all of the information contained in this literature.

Historical Background

It is to Charles Darwin and his son Francis (1), that indebtedness is due for the subsequent discovery of plant hormones in the twentieth century. They predicted that plant growth is dependent upon the production and diffusion of some active material produced by the growing tips in plants. They proved this rather revolutionary concept experimentally in 1880 by cutting off the tips of plants and also by covering the tips of other plants with blackened glass tubes. This prevented the entrance of light to the tip of the plant. Although the rest of the plant was exposed to light it did not respond by bending towards the

light as did the control plants. Experiments were performed by placing plants in a box open on one side in front of a window. From their observations the Darwins concluded that tropistic growth curvature in the basal region, following illumination of the shoot tip, depended upon the transmission of some influence from the tip downward.

In 1910 Boysen-Jensen (2) demonstrated that the influence is material, since it can cross an incision but cannot pass through a mica barrier. He demonstrated this by inserting a sheet of mica into the incision made on the tip of the coleoptile of oats. This inhibited the transmission of the stimulus when the incision was situated on the shaded side. His assumption became a certainty when he cut off the tip of the coleoptile entirely about one centimeter from the top, removed the primary leaf inside, placed a drop of gelatin on stub of the coleoptile, and replaced the coleoptile tip in its former position. When the tip was unilaterally illuminated, a distinct stimulus transmission took place and a strong curvature occurred in the dark base. These experiments showed that the transmission of the phototropic stimulus takes place on the shaded side of the coleoptile and that the stimulus can be transmitted across a wound under favorable conditions.

The experimental study of plant growth initiated by Boysen-Jensen was soon extended and refined by Paal (3). An important development from this work was the observation that if the decapitated tip was replaced asymmetrically,

the shoot curved so that the side in contact with the displaced tip was convex. But the observed curvature, caused by the differential growth of the coleoptile, occurred in total darkness. Paal correctly inferred that normal growth is regulated by the symmetrically distributed diffusion of a material substance synthesized by the tip. Curvature, he reasoned, was caused by some inequality of distribution associated with the action of light or gravity. Several hypotheses have been proposed to explain this unequal distribution. One suggests that the substance has a negative response to light whereas another indicates that a chemical reaction takes place when light is present whereby the growth regulator is inactivated on the sunny side of the plant. Regardless of which explanation is accepted, undoubtedly, a higher concentration of the substance is produced on the side of the plant furthest from the light source. Therefore this region would grow more than the region nearest the light source causing the plant to grow towards the light.

A further step in the analysis of plant growth regulation was contributed by F. W. Went (4) in 1926. He permitted decapitated tips to remain in contact with agar for some time and found that when such agar blocks were placed on test stumps the usual curvatures were observed. These were in no way distinguishable from those caused by side illumination or asymmetric tip replacement. In fact, judging from the effects produced on the test stump, the

agar block was physiologically equivalent to a functional tip. The curvature was shown to be proportional to the amount of material present in the agar; that is, it was proportional both to the number of tips placed on a given block and to the duration of contact.

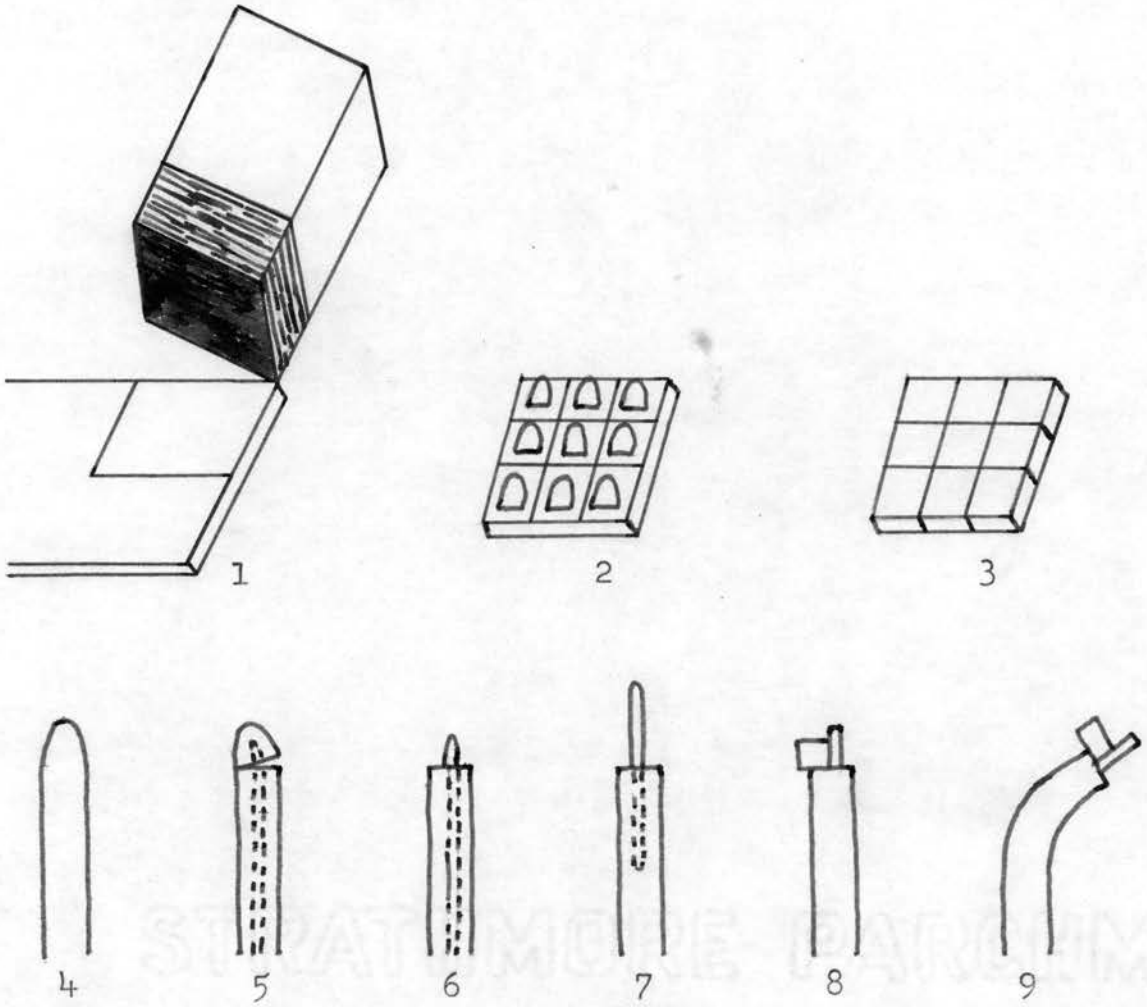

Figure 1. Schematic representation of the method for analyzing growth substances. From Went (4). 1. Stamping out agar plate from an agar film. 2. Placing coleoptile tips on the agar. 3. The agar plate divided into twelve blocks. 4. Unilateral incisions of the coleoptile. 5. and 6. Removal of the tips. 7. Pulling out the primary leaflet. 8. Agar block placed on one side. 9. Resulting curvature.

Research continued to unveil facts concerning plant growth regulators. In 1928 experiments, by Went (Fig. 2) and in 1933 by Van Overbeek (Fig. 3), indicated that unilateral light evidently causes the translocation of growth hormones toward the shaded side. Their findings helped to illustrate the hormonal explanation of phototropism (Fig. 4). Dolk, 1929; Boysen-Jensen, 1933; and Dijkman, 1934 (Fig. 5) showed that the growth hormone is transported to the lower side of the stem when a plant is placed in a horizontal position. These explanations confirmed the hormone explanation of geotropism by Cholodny (Fig. 6). Possibly it should be noted again that there are other hypotheses for the explanation of the phototropic response to light, namely, a chemical reaction, when light is present, whereby the growth regulator is inactivated.

Progress continued when in 1934 Kögl and co-workers isolated auxin a, auxin b and indoleacetic acid and characterized them chemically. Thimann extracted indoleacetic acid in 1935 almost simultaneously with Kögl. Zimmerman and Wilcoxon discovered several synthetic substances with hormone activity in 1935. During the years that followed it was generally believed that auxin a and b were the growth hormones in higher plants, and IAA a product of only the lower plant forms. However, when IAA was isolated by several investigators from several species of higher plants, opinions were reversed to the belief that IAA was the major, if not the only, auxin hormone in all plants.

Some investigators question whether auxin a and b even exist in plants. The question remains unsolved.

Availability of Reference Material

A search for laboratory exercises dealing with plant growth regulators for the high school revealed only one source. The Turtox General Biological Supply House has written two service leaflets on plant growth regulators which are sent free to all high school biology teachers: Turtox Service Leaflet No. 47, "Plant Experiments with Gibberellic Acid" and Leaflet No. 54, "Plant and Animal Hormone Experiments". These leaflets may be secured by writing to: Turtox Service Department, General Biological Supply House, 8200 South Hoyne Avenue, Chicago 20, Illinois.

Justification of Problem

Experiments dealing with plant growth regulators can be carried out on the high school level during regular biology laboratory periods plus being ideal for student projects. These experiments can be relatively simple and it is not necessary to resort to extensive gadgetry or complex equipment. Because of the vast numbers of plants, it is possible for students to do original research experimentation. It will provide a challenge to them to use their imagination for the various ways to conduct their experiments, instead of employing the "cook-book" method, and will train them in the art of observation.

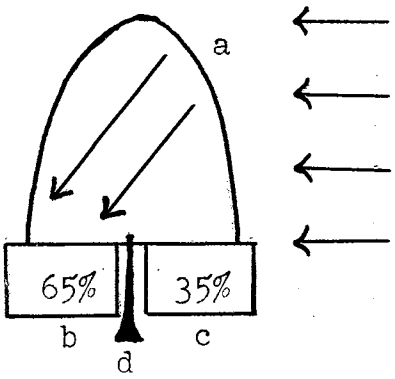

Figure 2. Differential concentrations of growth hormone by unilateral light. From Went (5). When unilateral light falls upon an excised Avena coleoptile tip, a, placed in contact with two agar blocks, b and c, separated by a razor blade, d, growth hormone is more concentrated toward the shaded side; block b receives 65 percent and block c 35 percent of all the recoverable growth hormone.

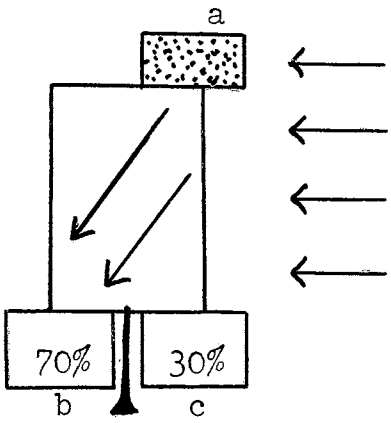

Figure 3. Differential concentrations of growth hormone by unilateral light. From Overbeek (6). An agar block, a, containing growth hormone is placed upon the upper cut surface of a Raphanus hypocotyl segment standing upon two plain agar blocks, b and c. Exposure to unilateral light produces differential concentrations of growth hormone on the shaded side; the recoverable portion is present in the two blocks as indicated.

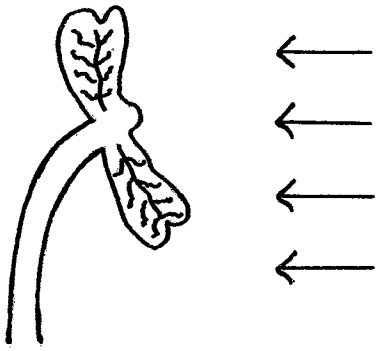

Figure 4. Hormone explanation of phototropism. The growth hormone is displaced by unilateral light into the shaded portion of a hypocotyl, petiole, or similar organ. Its presence in greater concentration promotes growth more rapidly there, and the organ bends toward the light.

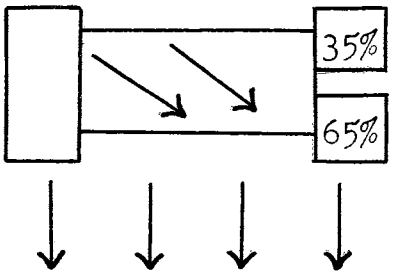

Figure 5. Growth hormone transported to lower side when plant is placed horizontally. From Dijkman (7). Growth hormone supplied in agar to the cut apex of a segment of Lupinus hypocotyl, placed in a horizontal position, is transported toward the lower side of the morphological base.

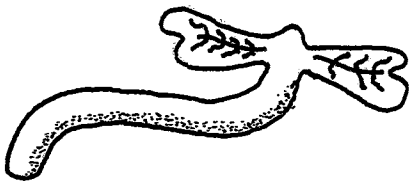

Figure 6. Hormonal explanation of geotropism. From Cholodny (8). Tropistic bending results from translocation of the hormone to the lower side of the plant axis. The shoot curves upward because its growth is promoted by the hormone, and the root turns downward because its growth is inhibited by the hormone.

TERMINOLOGY, DEFINITIONS AND CLASSIFICATION

The concept that normal plant growth is controlled by a material, translocatable-correlation agent was formulated by Paal in 1919. This hormonal concept became concrete with an extraction of the active substance, followed by isolation and characterization, in the early thirties, of three growth hormones, or auxins, from plant sources.

Throughout the field of plant growth research such terms as growth hormone, growth regulator, growth substance, phytohormone, auxin, and formative substance have been used with a great deal of synonymity and looseness. Recognizing the impediment inherent in confused and overlapping terminology, redefinitions have been suggested by Thimann (9) and by van Overbeek (10). The former defined phytohormone as:

"an organic substance produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production, and active in minute amounts".

Van Overbeek considered this definition impractical on two bases: first it is difficult to ascertain whether or not a substance is naturally occurring and secondly, he asks how far from the site of production must the substance act in order to be hormonal. He therefore suggested broadening the hormone definition to "an organic regulator of physiological processes".

The term hormone has a definite classical connotation of correlative function. It has no doubt been a mistake to refer to synthetic substances as hormones or auxins simply because they induce physiological responses similar to those of the natural hormones. It seems as though there should be some distinction between the two. Of course, it has been difficult to establish many organic compounds as natural hormones since they occur in such minute quantities in plants. This makes the extraction of them difficult and in most cases, if they are found in some plants they do not exist in others.

This complex problem would not be studied extensively by a student until he was quite advanced in college in the field of plant physiology. Therefore, this problem need not be a stumbling block for the study of these compounds in high school. The high school student will be primarily interested in the effects produced by these compounds and not by the part they play in metabolism. Although he should know that some are produced by plants and others are produced synthetically and that in many cases they produce similar effects. This is one more example that reveals the ingenuity of man to control his environment.

The following classification of plant growth regulators has been set up on the basis that either the compounds resemble one another structurally or the effects which they produce are similar. No attempt has been made to include

all compounds that have regulatory effects on plants in this list.

PLANT GROWTH REGULATORS

I. B-Vitamins
   - Thiamine
   - Nicotinic acid
   - Pyridoxine

II. Auxin-type regulators
   - Indoleacetic acid (IAA)
   - Napthaleneacetic acid (NAA)
   - 2,4-dichlorophenoxyacetic acid (2,4-D)
   - 2-methyl-4-chlorophenoxyacetic acid (MCPA)
   - Methylesternaphthaleneacetic acid (MENA)
   - 3-indolebutyric acid
   - 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)
   - 4-parachlorophenoxyacetic acid (4-PCPA)

III. Non-auxin type regulators
   - Isopropyl phenylcarbonate (IPC)
   - Ethylene
   - Ethylenechlorohydrine
   - Thiourea
   - Maleic hydrazide (MH)

IV. Urea derivatives
   - Diphenylurea
   - Chloromethyl urea (CMU)

V. Adenine derivatives
   - Kinetin
   - Adenine
   - 6-Benzylamino purine

VI. Unsaturated lactones
   - Coumarin
   - Scopoletin
   - Protoanemonin
   - Parascorbic acid

   - Gibberellic acid (?)

VII. Cyclopentadiene derivatives
   - Cyclopentadiene
   - Hexachlorocyclopentadiene

EFFECTS PRODUCED BY PLANT GROWTH REGULATORS

Under normal conditions the concentrations of the plant growth regulators present in plants allow us to classify them into two classes, (1) those that stimulate shoot growth and inhibit root growth and (2) those that inhibit shoot growth and stimulate root growth. An explanation is necessary to justify this classification. We shall refer to them (1) as a stimulator and (2) as an inhibitor. The apparent contradiction will be satisfied with the following explanation.

The plant growth stimulators are necessary for the growth of both the shoots and the roots. The greater the concentration of it, the greater is the growth of the shoot, unless it reaches toxic levels. The stimulator is also necessary for root growth but the optimum level is far below that of the shoot. The concentration necessary for shoot growth is therefore inhibitory to the roots. That is the situation in nature. Before continuing to explain about the inhibitors it is necessary to understand how the stimulators function. The most probable explanation is that the growth stimulators are effective only when they are bound to certain active sites on the receptors of protein nature. Then they are able to produce their effects in the metabolism of the plant.

Plant growth inhibitors may be divided into two groups. (11). The first group being the antiauxins, those which inhibit competitively the action of auxins. The antiauxins affect plants by competing with the stimulators for the active sites on the protein receptors. An antiauxin attached to a receptor forms a compound unable to function in metabolism. Therefore, in the entire plant the antiauxins would reduce the amount of attachments possible to the growth stimulators. This would of course reduce the amount of the complex formed between the stimulator and the protein receptors. The reduction of this complex in the shoot would inhibit shoot growth whereas in the root the reduction would stimulate its growth since minimum quantities of this complex in the roots provides maximum growth. The second group, known as inhibitors, limit growth either by being toxic to the plant or possibly they act in some regulatory manner. There is evidence that otherwise uncontrolled growth may be regulated by these growth inhibitors. Coumarin and scopoletin belong to this group.

In many texts you will find that the term "stimulator" is replaced by "auxin" and the term "inhibitor" is replaced by "antiauxin". However, auxins, by definition, are naturally occurring compounds found in plants that cause cellular enlargement. Since this would bar many of the plant growth regulators from the discussion, this terminology was not used. In fact, in actual practice more work is being done with the plant growth regulators that are neither

auxins or antiauxins. This is primarily because auxins and antiauxins exist in minute quantities in plants and they are therefore difficult to extract.

It has been suggested that growth is a balance between stimulators and inhibitors. During the formative period the stimulators would be in excess, whereas, the mature plant would contain excess plant growth inhibitors.

The following morphological effects of auxin function has been cited by Gordon (12). The same effects can also be produced by plant growth stimulators or inhibitors that are not auxins. He stated that their functions to a great extent are manifestations of growth responses.

1. The application of high concentrations of auxin to young tissue causes swelling, a reaction that may entail both cellular enlargement and division.

2. The role of auxin as an evocator is likewise manifest in the initiation of root primordia. Physiologically low concentrations of auxins likewise induce cell division in roots. However, almost any living plant tissue -- cambium, epidermis, pericycle, endodermis, cortical parenchyma, pith rays -- will form roots by the interaction of a suitable gross nutritional level and raised auxin level.

3. There are numerous indications that auxin has some role in floral induction. For example, it is well known that the direct application of auxin to the pineapple will rapidly convert the vegetative apical meristem to a

floral apex. Yet in a number of other plants, auxin treatment will delay or inhibit flowering. Also, mature, seedless fruits have been produced on numerous plants by direct application or injection of various pure auxins to unpollinated pistils.

That applied auxin can be substituted for pollination in ovary development suggested at first that the pollen tube serves not only as the means of gamete transfer, but also as the source of auxin required for fruit setting. However, the pollen and ovary auxin levels aren't sufficient to account for all the free auxin in the ovary following pollination. Apparently auxin synthesis in the ovary is activated by fertilization.

4. Another manifestation of the auxin response after fertilization is the inhibition of abscission layer formation in fruits like apple, and hence, the organ is retained on the stalk. An essentially similar auxin controlled process is the abscission phenomenon of petioles and flower stalks, which is likewise directly inhibited by pure auxin supplement.

5. Auxin also participates in the quantitative determination of leaf form. Auxins stimulate vein development. Adenine controls mesophyll growth.

6. Plant growth regulators also inhibit or stimulate seed germination.

7. As mentioned previously auxin will accelerate root growth, but only at extremely low concentrations.

Normally, all of the concentrations that stimulate shoot growth are inhibitory to the root.

8. According to the classical theory, the phenomenon of apical dominance also appears likewise to be auxin-mediated. Terminal buds tend to suppress lateral buds basipetal to them. It was believed for many years that auxin functioned as the primary agent in this suppression. However, recent experiments have indicated that apical dominance in Coleus is not controlled by auxin from the apex. Jacobs et al (13) substituted IAA in lanolin for the top of the main shoot of Coleus and found that the lateral buds basipetal to the main shoot were not suppressed. Therefore, it appears that possibly auxins do not control apical dominance, however, more experimental evidence is necessary before any conclusions should be drawn.

EXPERIMENTS DESIGNED FOR HIGH SCHOOL BIOLOGY LABORATORY PERIODS AND STUDENT PROJECTS

Forword

The following experiments are by no means the only ones suited to the high school. The ones included in this report were modified from experiments in the manuals listed below and merely serve as an introduction. Many new ideas for experiments with plant growth regulators can be gained from Turtox Service Leaflets, which have been previously mentioned. Other experiments of educational value are also contained in the following manuals: Plants in Action, by Machlis and Torrey, W. H. Freeman and Company, San Francisco, 1959 and Agriculture Handbook No. 126, Test Methods with plant-regulating chemicals, Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. (40 cents).

Sources of Materials

While it is impracticable to provide a complete list of dealers from which plant growth regulators may be purchased, this partial list is furnished for the users information.

Eastman Kodak Co.,
Chemical Division,
Rochester 4, N. Y.

Fisher Scientific Co.,
1458 North Lamon Avenue,
Chicago 51, Ill.

General Biological Supply House,
8200 South Hoyne Avenue,
Chicago 20, Ill.

Nutritional Biochemicals Corporation,
21010 Miles Avenue,
Cleveland 28, Ohio

E. H. Sargent Co.,
4647 West Foster Avenue,
Chicago 30, Ill.

Will Corporation of Maryland,
5 North Haven Avenue,
Baltimore, Md.

Experiment One

Geotropism: Soak corn seeds overnight in distilled water. Select 6 seeds, place them in a Petri dish with embryos down, and arrange them across the center of the dish with all the pointed ends oriented in one direction. Using moist filter paper slightly larger than the dish, and then soaked paper toweling, cover the seeds and press them firmly into place so that they will not change position. Prepare a second Petri dish in exactly the same way. Place the Petri dishes on edge with the points of the seeds down. Mark the upper edge of the bottom Petri dish, not the lid, of each dish with a wax pencil. Transfer Petri dishes to the dark at 25°C making sure that Petri dishes are on edge with a mark on top, therefore denoting that the pointed ends of the corn are pointing down.

Forty-eight hours later observe the seeds. Carefully excise the terminal two millimeters of all of the roots in one dish with a razor blade. Arrange all the roots so that they are parallel, replace the moist filter paper, and turn the dish 90° so that the roots are now horizontal. Arrange the roots in the other dish in the same manner, but leave them intact. Rotate 90° to make the roots horizontal. Return the dishes to the dark at 25°C. At the beginning of class the next day, observe the two sets of roots and record the results in the form of a continuous growth diagram.

Experiment Two

Stimulating root initiation: In commercial practice, most plant propogation is by stem cuttings. Many plant growth regulators accelerate root development on such cuttings. Therefore, they have found widespread use among nurserymen, florists, horticulturists, and home gardeners.

Soak thirty seeds of common garden bean for about one hour in tap water in a beaker. Plant the seeds well apart and about one-half inch deep in a small, paper-lined flat of sand. Water the sand thoroughly; then germinate in the dark at 25°C for five days, until the hypocotyls begin to show. Transfer the flat to the greenhouse or window sill, and grow for an additional seven days, until the plants have formed a pair of simple leaves.

Completely cover the outside of four half-pint screw-cap jars with heavy aluminum foil. Tie a tag round each jar neck with string. Fill each jar with 200 ml of one of the following solutions, and label:

1. Distilled water.
2. Quarter-strength Hoagland solution with added micronutrients (See page 22 for preparation).
3. As in No. 2, plus 0.1 mg indoleacetic acid per liter.
4. As in No. 2, plus 1.0 mg indoleacetic acid per liter.

Cover each jar with the five-holed tin lid, and screw it in place.

With a sharp razor blade, excise each bean plant at the level of the earth, remove both cotyledons, cut the hypocotyl 5 cm long, as measured from the point of cotyledon attachment to the cut base. Immediately put the hypocotyl through one of the holes in the lid and into the solution, with the pair of leaves projecting above the lid and the cotyledonary stumps below the lid. Repeat the procedure, one plant at a time, working rapidly to avoid drying of the plants, until five plants have been placed in each jar. Select plants as similar as possible. Place the jars in a row on the shelf above the laboratory bench. Do not place jars in the greenhouse or on a window ledge, since they would suffer excessive water loss.

One week later, measurements are made. Unscrew the cap, remove the lid, and cut each hypocotyl just above the cotyledonary stumps to facilitate handling. Make the following measurements on each hypocotyl.

1. Number of rows of later roots.

2. Number of lateral roots (longer than 1 mm) in each row.

3. Number of lateral root primordia (shorter than 1 mm) in each row.

4. Length of lateral roots in mm.

Determine the averages for each treatment, and summarize the results.

Preparation of Hoagland solution. Add the following solutions, one at a time, to 1870 ml of distilled water. 10 ml of 1 M $Ca(NO_3)_2$, 10 ml of 1 M KNO_3, 4 ml of 1 M $MgSO_4$, 2 ml of 1 M KH_2PO_4, 2 ml of $FeCl_3$, and 2 ml of micronutrients. Total mixture should equal two liters. To prepare 1/4 strength Hoagland solution use only 1/4 of the quantities of the solutions above, except for the micronutrients and $FeCl_3$ which should be full portions. Micronutrient solution should contain 2.86 g of H_3BO_3 (boric acid), 1.81 g of $MnCl_2 \cdot 4H_2O$ (manganese chloride), 0.11 g of $ZnCl_2$ (zinc chloride), 0.05 g of $CuCl_2 \cdot 2H_2O$ (copper chlorite), and 0.025 g of $Na_2MoO \cdot 2H_2O$ (sodium molybdate) per liter.

Experiment Three

Apical dominance: Apical dominance is controlled by the terminal bud, which produces large amounts of auxins. These auxins promote development of a single main shoot axis, with the suppression of axillary buds. The following experiment illustrates how indoleacetic acid (IAA) will promote normal plant development even though the terminal bud is removed.

Plant 12 pea seeds, variety Alaska, in washed sand in a 4" pot, and place the pot in the dark at 25°C. Use a pot label to identify the plants.

On the 5th day after planting, cut off the shoots of two-thirds of the plants just below the last pair of leaves. Mark half of these with reinforcement rings (gummed reinforcement rings used to reinforce holes in notebook paper), and apply lanolin paste containing 400 ppm IAA to the decapitated surface; to the other half apply plain lanolin paste. The intact plants should be left for comparison. Return the pot to the dark. The lanolin paste in each treatment should be renewed two or three times a week.

On the 14th day after decapitation, measure to the closest mm the length of the axillary buds that have developed in the axils of the cotyledons in the three sets of plants. Measure in mm the diameter of the stem at the level of the cut surface in the decapitated and intact plants.

Experiment Four

Overcoming apical dominance: Maleic hydrazide has been used as a chemical means of overcoming apical dominance, and thus as a substitute for the manual pinching out of chrysanthemums and other flowers when a much-branched plant is desired. As a growth inhibitor it can be used to slow down the growth of lawn grass and to prevent sprouting of tubers, onions, and similar storage structures.

Transplant two tomato seedlings four-inches in height to each of two 4" pots, water them, and allow them to grow for an additional week in the greenhouse. Label each pot with your name.

On the 7th day, using an atomizer, spray one entire plant with a sufficient volume of 0.4% aqueous solution of maleic hydrazide to thoroughly wet all surfaces of the plant. Continue the untreated plant as the control.

Observe the two plants over the period of the next four weeks. At the end of the 4th week, make the following observations: (1) total height of each plant in centimeters; (2) development of axillary buds and leaf form; (3) color and appearance of plants.

Experiment Five

Fruit-set: The initiation of growth of the fruit, technically termed fruit-set, is controlled by plant growth regulators and can be caused artifically without pollination by direct spray application of synthetic regulators to the plant at the appropriate stage of flowering. The production of fruit without pollination is called parthenocarpy. Fruits produced in this manner do not produce seeds.

α-naphthaleneacetic acid, p-chlorophenoxyacetic acid, and β-naphthoxyacetic acid are widely used commercially to induce fruit-set. Artificial means has been widely used especially in tomato plants. Workers in horticulture have also been successful in obtaining seedless fruit in cucumber, pepper, squash, blackberry, grape, and other plants with the use of plant growth regulators.

A. For tests of limited duration (14 - 20 days): Grow tomato plants in 4 to 6 inch pots set side by side in rows 4 inches apart, and select those of uniform size which have developed two open flowers on the first cluster.

B. For prolonged tests involving maturation of the fruit (35 - 45 days): Grow the plants in 10 to 12 inch pots, or grow them in ground beds. In either case select uniform plants that have developed 2 open flowers in the first cluster. The plants should be at least 12 inches apart, in rows 2 feet apart.

Prepare a lanolin-Tween 20 paste containing 1% of the beta-naphthoxyacetic acid, and apply a narrow band of the mixture around the stalk (peduncle) of the first flower cluster and about 1-2 cm. from the main stem of the plant. Leave an additional row of plants untreated to serve as controls. (To prepare mixture place 25 mg. of the beta-naphthoxyacetic acid in a vial and add 14 drops of Tween 20. Stir to dissolve the chemical, and add 2 g. of lanolin. Melt the lanolin by placing the vial in warm water (not over 55°C.) for a few minutes. Remove the vial and stir the mixture thoroughly until it reaches room temperature and becomes semi-solid.) As a flower cluster is treated add a label.

A. Plants used in tests of limited duration: Count the number of flowers and the number of fruits that set (remain attached to the plant) per cluster within 14 to 20 days after treatment, or before the plants become excessively pot bound. Determine the percentage of fruit that set. Cut the green fruits midway and at right angles to their axes, and record the relative amount of gelatinous pulp and the relative numbers of seeds present.

B. Plants used in a prolonged test: Record the data as described above and, in addition, record the number of days required for the fruit to develop a pink or red color.

If needed, use the second and third flower clusters on each plant to repeat the earlier treatments. Plants in

ground beds may develop 5 or 6 flower clusters that are suitable for treatment.

Experiment Six

Germination: The seeds to be treated are placed overnight on filter paper that has been moistened with a gibberellic acid solution. Strengths of solutions may vary so as to test their effects at different concentrations. (5/8 gram of Turtox Gibberellic Acid "10" Powder dissolved in four ounces of water will give the proportion of 10 ppm. A solution containing 100 ppm of active gibberellic acid may be prepared by dissolving 5/8 gram of Turtox Gibberellic Acid "100" Powder in four ounces of water.)

The next day the seeds should be planted in moist sand or vermiculite. In general, the treated seeds germinate better and seedlings emerge more rapidly than in untreated seeds. However, there are many variations, and excessive gibberellic acid applications produce numerous seedling abnormalities.

Experiment Seven

Internodal stimulation: Most plants are affected by gibberellic acid in their initial growing period or when buds are forming. Plant length is usually stimulated; often the plants increase three to five times in height within two or three weeks. Herbaceous plants are usually responsive to 10 ppm, whereas, woody plants require 100 ppm.

Method of Application. Spray the solution with any small household or garden sprayer, wetting stems as well as upper and lower surfaces of foliage thoroughly to the point of run-off. With some plants a single application is sufficient, but if no reaction is noted in ten days, repeat the application at ten-day intervals until growth response is noticed. Repeated applications may result in excessive growth -- leading to the development of plant monstrosities. Repeated applications have produced cabbage plants ten feet in height and early flowering (But no marketable heads!).

Caution: because of the inhibitory effect on root formation, it has been found important that the root system of the experimental plant be liberally treated with fertilizer.

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