How-To-Do-It

Some Plant Hormone Investigations That Work

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Wouldn't it be nice to find some inexpensive laboratory investigations that are easy to set up and maintain, applicable at several levels of instruction and useful in several biology courses? If these investigations used living organisms showing constant directed change, illustrated both practical applications and creative thinking and were conducive to statistical analysis then most teachers would probably be interested.

This article describes some plant hormone investigations that possess this type of potential. These investigations are usually performed simultaneously with discussion of growth regulating substances in the lecture portion of my course in plant biology. They could be set up prior to the lecture explanation and used as discovery exercises, or they could be performed in an introductory level course to illustrate the control and experimental conditions. What follows is the description of the investigations in the form given to students. They receive all of the information up to the section on *Additional Information.*

Hormonal Regulation of Plant Development

Introduction

The final form of a plant is a result of complex interaction involving the plant's genetic information and the modifying effects of its environment. Plant growth regulators are organic molecules that play a major role in modifying plant growth and development. A subgroup of plant growth regulators are the plant or *phyto*hormones. Like hormones in animal systems, they are produced in minute amounts and transported (translocated) to target sites where they cause some response. As cells differentiate and tissues and organs develop specific form and function, hormones provide a means of precise coordination between these individual parts during each stage of the organism's growth.

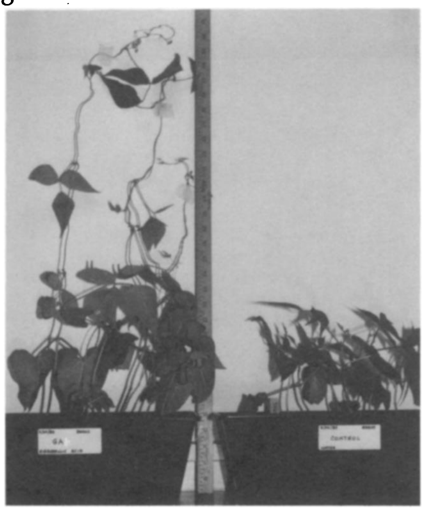

Figure 1. Effect of Gibberellic Acid Treatment. Bean plants on left show the effect of gibberellic acid treatment after three weeks. Plants on the right are the untreated control. Photo by Donald E. Brott

The control of differentiation and growth resides, ultimately, in the DNA of the nucleus. Nuclear information determines the production of hormones and other regulatory chemicals and, in turn, may be subject to their modifying effects.

There are five widely accepted groups of plant hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. Research in this area is complicated because these phytohormones interact with each other in a number of different ways dependent on concentration, target site, species involved, developmental stage and environmental factors—to name but a few. Unlike animal or bacterial studies, there are usually no type species which may serve as general models for the effects of the phytohormones. Additionally, there are many other phytohormones restricted to specific plant groups and artificial plant regulators which may also fall into this classification.

The following investigations provide some insight into the effects of certain environmental and chemical factors on the patterns of plant growth and differentiation.

I. *Effect of Gibberellic Acid on Plant Growth.*

Select 20 plants (either pea or bean) which have been growing in soil for approximately two weeks. Divide the plants into two equal groups. Label one group "Control" and the other "Gibberellic Acid" or "GA3". Label each individual plant within each group with a number and the date or use a floor plan method. Measure and record the height of each plant (in mm) from the cotyledons to the tip of the shoot apex.

Apply one drop of gibberellic acid to the shoot apex of each plant in the tray labeled "Gibberellic Acid" and apply one drop of distilled water to the shoot apex of each plant in the Control group. BE SURE TO USE SEPARATE MEDICINE DROPPERS FOR EACH SOLUTION. BE SURE TO WATER THE ROOT SYSTEMS OF THE PLANTS WITH EQUAL AMOUNTS OF TAP WATER. Follow this procedure weekly, and record the height of each plant in each group and the general appearance of all the plants. When the experiment is concluded, plot the data (height vs. time), and determine whether growth response obtained with Gibberellic Acid is significantly different from that of the controls.

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II. *Inhibitory Effects of Synthetic Plant Regulators.*

Succinic acid 2,2 dimethyl hydrazide or N-dimethylamino-succinamic acid (commonly known as Alar or B-Nine) is a synthetic plant regulator with effects directly opposed to the gibberellins and IAA. Choose five healthy tomato or marigold plants that have been growing in soil for three weeks to serve as your experimental group and five others to serve as controls. Label, measure, and record the data for each plant in each group. Spray each plant in the control group with distilled water once a week for three weeks. Spray each plant in the experimental group with B-Nine once a week for three weeks. USE SEPARATE ATOMIZERS FOR EACH GROUP. BE SURE TO WATER THE ROOT SYSTEMS OF THE PLANTS WITH EQUAL AMOUNTS OF TAP WATER. During this time, note and record changes in height and general appearance of all plants in each group. Plot individual and collective differences and determine if height differences are statistically significant.

A useful follow-up is to plant the experimental and control groups in a garden or greenhouse and determine how long it takes for the experimental group to catch up to the control group.

III. *Effect of Varying IAA Concentrations on Root Growth.*

Choose six healthy tomato plants that have been growing in soil for approximately three weeks. Carefully remove each plant from the soil and gently wash the root system with tap water. Measure each plant and record its general appearance. Place each plant in a separate tube by threading the wet root system through the hole in the cork stopper. Fill each tube with the appropriate concentration of IAA so that the entire root system is covered. Place aluminum foil or a similar material around the bottom of the tube to prevent the entrance of light. Place a sixth plant in a tube of tap water as a control. Observe root growth for the next three weeks.

Your instructor will show you how to compute the differing concentrations to be used (0.01, 0.10, 1.0, 10.0, 100.0 mg/liter).

Set up another set of tubes, using the same concentrations, with ACETIC ACID. Compare the results to those of IAA and control. Explain the results.

Additional Information

There are some general precautions to be considered when using any plant growth regulator and some specific considerations for each one used.

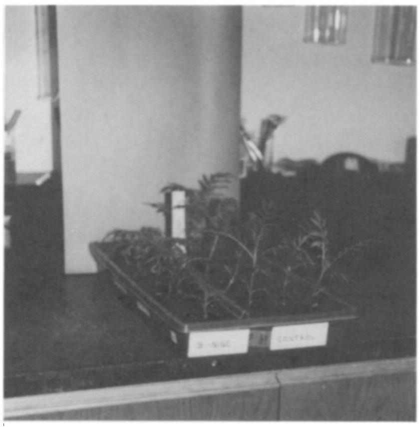


Figure 2. Effect of B-Nine Treatment. Marigold plants on the left have been treated with B-NINE for three weeks and illustrate reduced height due to disruption of IAA production. Plants on the right are the untreated control. Photo by Donald E. Brott

Avoid contact with the skin, eyes and clothing. Hands should be washed thoroughly after using any of these chemicals. Keep all formulations refrigerated when not in use. If solutions are to be used for several weeks, make new ones about every two weeks. Be sure that all glassware is thoroughly washed and rinsed after contact with these chemicals.

All hormones and plant regulators are available from Carolina Biological Supply Company. For investigation I, the gibberellic acid solution is made by dissolving 100 mg. of gibberellic acid powder in 2.5 ml of 95% ethyl alcohol. Dilute the resulting solution with distilled water to make 1 liter. This will give a solution of 100 mg./liter gibberellic acid. Almost any species of pea, bean or sunflower will work in this investigation. If you wish to show growth acceleration, "Little Marvel" dwarf peas are suggested. We have been successful with pole beans (see Figure 1). Since gibberellic acid may retard root growth, be careful not to spill any on the soil. The developing leaves of plants treated with gibberellic acid will start out as pale green and darken after the growth spurt is over.

B-Nine disrupts the synthesis of IAA and will tend to reduce internodal elongation. We have used several different species of tomato and marigold plants successfully (see Figure 2). When spraying the plants, cardboard shields should be used to prevent drifting of the solution. Wet the entire plant but try to prevent dripping or runoff from the plant. To prepare the B-Nine solution, dissolve one gram of B-Nine powder in 99 ml of distilled water. This provides a solution of 10,000 ppm which is equivalent to a 1% solution. A wetting agent, such as Tween 20, should be added at a rate of one drop/50 ml of solution.

The purpose of investigation III is to show the inhibitory effects of high IAA concentrations on root growth. While high concentrations of IAA will stimulate root formation, they tend to inhibit root growth in established roots. We have had good success using tomato seedlings (see Figure 3). Test tubes may be used to hold the plants. We use small vials which mea-

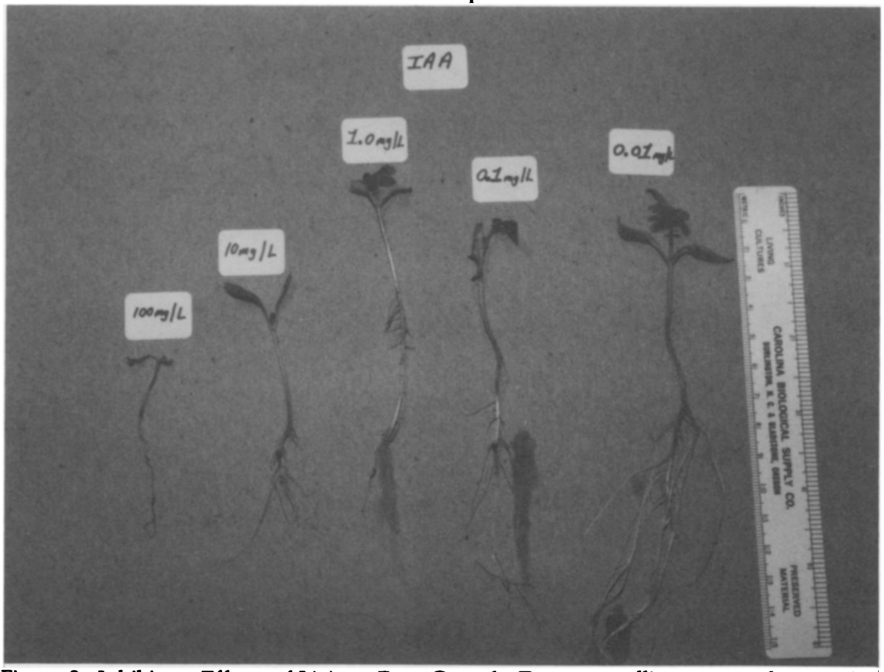
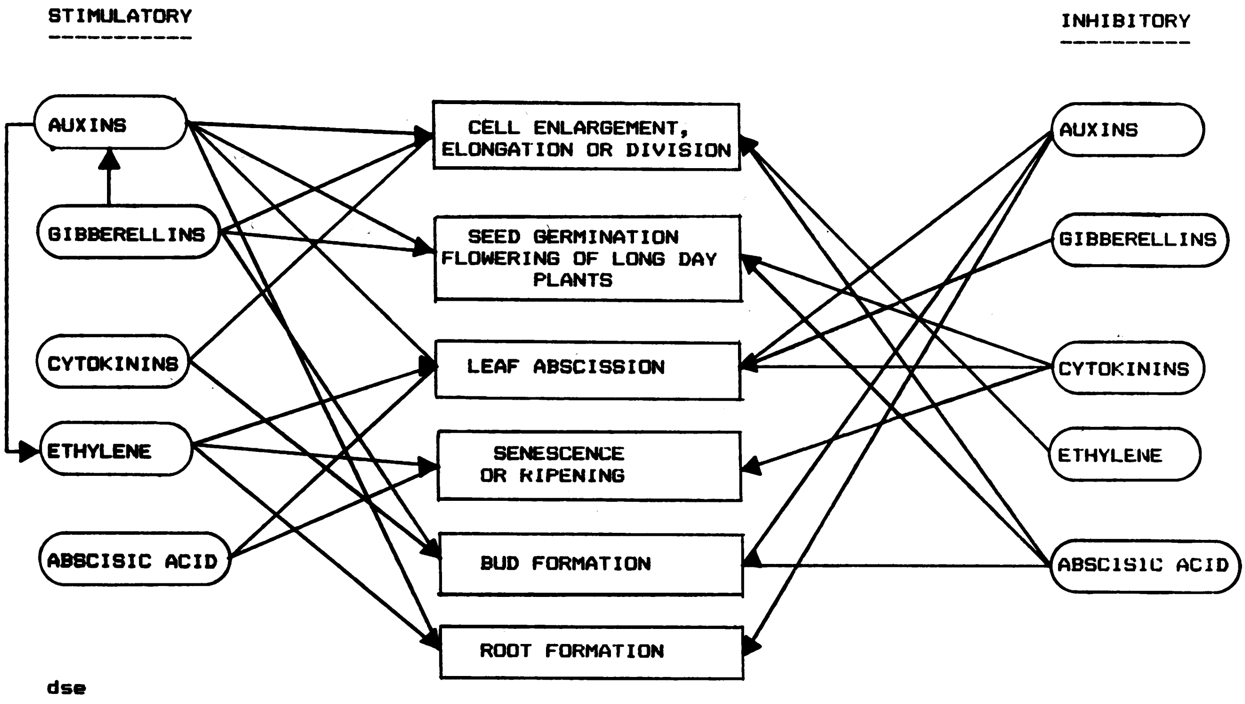


Figure 3. Inhibitory Effects of IAA on Root Growth. Tomato seedlings exposed to varying concentrations of IAA show reduced root growth. Plant exposed to 100 mg/liter concentration has died. Photo by Donald E. Brott

Figure 5. Generalized Reactions of the Major Plant Hormones.
Virtually all hormonal reactions are affected by environmental stimuli such as light, temperature, water and carbon dioxide availability, pH, and presence of specific minerals or organic compounds.
Reactions may differ from species to species, from tissue to tissue. Hormonal concentration often determines if the effect is stimulatory or inhibitory.



sure 83 mm by 22 mm. These vials require a #3 cork with a hole drilled by a #2 cork borer. If the plant is too small for the opening, wrap it with nonabsorbent cotton. Replace solutions as necessary. To prepare the IAA solution, dissolve 100 mg of IAA in 1.5 ml of absolute ethyl alcohol and add approximately 900 ml of distilled water. Cautiously warm the mixture on a hot plate to evaporate the alcohol and then dilute with distilled water to make one liter. This provides a basic stock solution of 100 mg/liter. Each new dilution should be made from the preceding one. You'll see your best results near the end of the second week. If you plan to analyze your results statistically, be sure that you have a sufficiently large sample number. You will need to run several sets of each investigation to ensure statistically valid results.

Figure 5, Generalized Reactions of the Major Plant Hormones, shows some of the interactions among the five major plant hormone groups. It illustrates the concept of homeostasis and is given to students when we are discussing plant growth regulators in lecture.

Some Overlooked Objectives and Outcomes

It has been suggested (Mills 1981) that one major advantage of laboratory investigation is that it not only enables students to learn biology by doing, but also encourages creativity, objectivity, thoroughness and precision. I would suggest that other outcomes also occur while doing these investigations. Students learn the advantages and limitations of controlled experimentation. In addition, they learn the benefits of teamwork, record keeping, time budgeting and following directions. Powers of observation are trained, handling and measurement techniques improved. Students find graphical representation of data, data analysis and statistical analysis necessary and useful. In addition, the instructor is challenged to keep abreast of information regarding plant growth regulators in order to answer the questions such investigations generate.

References

Abramoff, P., & Thomson, R.G. (1982). *Laboratory outlines in biology—III.* San Francisco: W.H. Freeman and Company.

Evert, R.F., & Eichhorn, S.E. (1976). *Laboratory topics in botany* (2nd ed.) New York: Worth Publishers, Inc.

Flagg, R.O., & Stone H.J. (1983). *Plant growth regulators.* Burlington, NC: Carolina Biological Supply Company.

Hereda, D., & Fieldhouse, D.J. (1970). Control of internal growth factors. *The Science Teacher, 37*(3), 83-85.

Lee, A.E. (1963). *Plant growth and development, A laboratory block.* Boston: D.C. Heath and Company.

Mills, V.M. (1981). The investigative laboratory in introductory biology courses: A practical approach. *The American Biology Teacher, 43*(7), 364-367.

Figure 4. Setup Used to Demonstrate Effects of Differing IAA Concentrations on Root Growth. Photo by Donald E. Brott