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Title of Study: BOTANICAL LABORATORY EXERCISES FOR THE ADVANCED HIGH SCHOOL BIOLOGY STUDENT

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Scope of Study: The purpose of this study was to compile a series of botanical exercises which the high school teacher may use to create interest in this field of science. Four major areas of botany are included with a variety of experiments within each. The subject range should be diverse enough to include the interests of most students. This paper is limited in that it is designed primarily for the advanced high school student of biology whose background includes general biology, chemistry and possibly physics. Some of the exercises require extensive time and apparatus which may be a limiting factor in some schools.

Findings and Conclusions: The botanical studies described herein should be of value in interesting the student in botany. Each exercise, properly prepared for and conscientiously conducted, should provide the student with a broader knowledge of a few of the areas of plant science. Some elementary research techniques will be employed and an interest may develop. With this background the student may possibly consider some field of botany as a career and pursue this course of study.

ADVISER'S APPROVAL James H. Tate

BOTANICAL LABORATORY EXERCISES FOR THE ADVANCED HIGH SCHOOL BIOLOGY STUDENT

By

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Report Approved:

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PREFACE

The purpose of this report is to better acquaint the advanced student of biology with botany as there seems to be little emphasis placed on this science in the high school today. It is hoped that by the use of some of the exercises presented by this paper the students will be motivated to a further study of plant science and will consider the career opportunities available in botany and botanical research.

The author wishes to express his sincere appreciation to Dr. L. Herbert Bruneau, Dr. Eddie Basler and Dr. John E. Thomas, under whose guidance this report was prepared. Additional gratitude is expressed to Dr. Basler for the use of communications from his personal file which appear in this report.

Indebtedness is acknowledge to Dr. James Zant, Director of the Academic Year Institute for High School Science and Mathematics Teachers and the National Science Foundation for their assistance.

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PART I

GENERAL INTRODUCTION

Mans primary interest in the world in which he lives concerns its relationship to himself. The basis of this relationship lies in the vegetation of the world, for without plants, no animal life could exist as plants provide the basic necessities for animal life. The study of this basic element of life is a science known as botany--a rather uncommon word in the high school biology course although the science of plants is taught to some degree. Although the majority of high school biology courses dealing with botany give precedence to morphology, anatomy and terminology of flowering plants, this field of science may be studied from a number of different aspects, each of which has been organized as a special phase of botany. This has been made necessary for purposes of advanced study because botany has become too vast a subject to be mastered in its entirety by any one person.

The curriculum, as set forth in most public high schools, shows a deficiency in the study of botany. For this reason, this collection of botanical exercises has been assembled for use by the high school biology teacher. An effort has been made to present some of the more ad-

vanced ideas as well as a challenge for the advanced student and introduce the aspect of botanical research by giving these students direct experience with a subject in which they are interested.

The advanced students, as referred to in this paper, are primarily those in the upper one-fourth of the class who exhibit an interest in science, possess the initiative and resourcefulness to assemble the necessary apparatus and do limited laboratory work without constant supervision. Their background should include general high school biology, chemistry and possibly physics as well as a limited knowledge of plant classification, structure and physiology. They should be familiar with the library and its facilities as a review of the available literature concerning each problem is important. They must also have a genuine interest in the project they undertake.

Before attempting any exercise proposed here the student should consider such factors as the time involved and the availability of apparatus. He should be willing to devote time to a literature review of the subject, to learn as much about it as possible, and be able to conduct the experiment with a minimum of help from the teacher.

The teachers role in this program is to encourage independent work and research. At times, however, some individual attention to more difficult procedures and interpretations must be given but the student is expected to handle the more elementary problems. The teacher

should attempt to develop in the student the capacity to think clearly, independently and logically about the problem, while he, the teacher, merely directs the basic ideas.

This paper is limited in that it is designed for the advanced pupil with an interest in botany and research. In addition, the necessary laboratory facilities may not be available in many small schools. Funds, for the purpose of buying miscellaneous apparatus, may not be available. A good library, which is essential for references, may not be accessible to many and some of the exercises will have seasonal limitations or the time available may not permit completion of some of the long range problems.

An appendix containing the necessary information concerning some of the more unusual materials and media has been included for the convenience of the student. However, the more common apparatus and reagents have been intentionally omitted for the purpose of giving the student some experience in developing these.

For those students who desire more information on the study of botany and the career opportunities possible in this field, a list of references is provided in the appendix.

An attempt has been made to prepare a collection of botanical exercises with enough variety of subject matter to include the interests of most high school students. It is the opinion of the author that the emphasis placed on physiology, ecology, pathology and genetics in this paper

is justified by the conviction that the study of these factors and the relatively outstanding results obtainable will be of most value to the student as far as interesting him in botanical research is concerned.

PART II

THE PHYSIOLOGY OF PLANTS

Introduction

The science of plant physiology has evolved from the very general treatment of plant growth and structure of the 17th century to the very concise and complicated science of today. No longer is the plant physiologist a strict botanist, for an understanding of the behavior of plants now requires a thorough knowledge of biochemistry and biophysics. Many of the old problems of plant structure and function remain today but are now being studied on the molecular level due to the ever increasing developments in biochemistry. This new approach to the science of plant physiology has increased the need for those who are willing to experimentally study plant structures and relate them to function.

In order to insure a supply of trained physiologists to explore these newer aspects of plants, the students of today must be attracted to this field. An effort has been made in this paper to set forth a few exercises in various phases of plant physiology which the high school teacher may use to create interest in this subject.

This part is divided into five major sections

representing various phases of plant physiology. Section 1 presents some of the problems concerned with the analysis of growth and development. Section 2 involves the inorganic ash present in plant tissue. Section 3 deals with the behavior and movement of water in plants. Section 4 presents some properties of cells and the nature of the cell and its function. Section 5 is limited to a study of the chlorophyll pigments.

The purpose of this part is to introduce the interested student to plant physiology by way of experimentation. In preforming the exercises the student will become acquainted with some of the problems and elementary techniques of this phase of botany and may interest him in doing advanced study and possibly research in this field.

Section 1--Plant Development

Exercise 1

Objective: To determine the region of growth of roots and stems.

Materials: Germinated peas, indelible marking ink, marking device, 600 ml beaker, glass pane to fit beaker, Petri dish, paper toweling, distilled water, rubber band, filter paper and metric rule.

Procedure:[1] A. Root Growth. Prepare a moist chamber by lining the sides of a 600 ml beaker with thoroughly moistened paper toweling. Completely cover a 3½" x 4" glass pane

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[1]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology, (San Francisco, 1956), pp. 16-17.

with coarse-grade filter paper soaked with distilled water. Select from the germinated pea seedlings one in which the root is fairly straight and at least 2 cm long. Wipe excess ink from the thread of the marking device. Lay the root on millimeter rule, and, starting at the tip, quickly mark 10 thin lines at 1 mm intervals with black marking ink. Place the seedlings against the moist plate with a rubber band supporting it just under the cotyledons. Repeat the operation until 5 roots are marked. Mark 2 more seedling roots with a single line 10 mm from the tip as control plants to test the effect, if any, of the marking procedure. Take care to avoid drying the seedlings during the marking. Support each root so that its whole length touches the moistened filter paper. Insert the glass plate supporting the seedlings into the beaker. Cover the beaker with the bottom of a 10 cm Petri dish and set aside.

At the end of 48 hours, measure the distance between the marks on each marked root. Average the lengths measured in the first interval behind the apex; do the same for all the other marked intervals. Measure the length of the control roots from the tip to the marked line. Plot the data on a separate sheet of graph paper and evaluate the results.

B. Shoot Growth. Using the same marking devices as in part A, carefully mark at 2 mm intervals with black marking ink the epicotyls of four of the 5-day-old etiolated pea seedlings (6 plants per 4" pot of sand, grown in the dark at 25°C). Hold the millimeter ruler vertical behind each

plant and mark from the tip of the epicotyl until 10 marks have been made. Mark 2 of the seedlings with a single line 20 mm from the tip; these will be control plants to determine the effect, if any, of the marking procedure. Place the plants in the dark.

After 48 hours, measure the distance between marks. Also measure the increase in length of the control plants. Determine the average elongation for each interval in the marked plants and plot these average lengths against the interval numbers.

Exercise II

Objective: To determine the effect of various wave lengths of light on cell expansion.

Materials: Cotton seed, sand, analytical balance, 12 Petri dishes, Shives nutrient solution (see appendix), facial tissue, red and blue cellophane.

Procedure:[2] Plant cotton seeds an inch apart in sand contained in shallow pans and germinate for 8 days at 30°C in the dark. At the end of 7 days remove cotyledons from the seedlings in a dark room and weigh in groups of 10. Weak red light may be used to carry out these operations. After weighing, place 10 cotyledons in each of 12 Petri dishes containing Shives nutrient solution and expose the cotyledons to the following light conditions: (a). Leave 3 dishes in the dark for 24 hours. (b). Expose 3 dishes to

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[2]Eddie Basler, Personal Communication.

red light for 30 minutes and leave in dark for 23.5 hours. (c). Expose 3 dishes to far-red light for 30 minutes and leave in dark for 23.5 hours. (d). Expose 3 dishes to red light for 30 minutes and far-red for 30 minutes then leave in dark for 23 hours.

After the cotyledons have been in the culture dishes for 24 hours, remove the cotyledons from the culture dishes, blot with facial tissue and determine the fresh weight.

Red and far-red light may be obtained as described in the appendix.

Exercise III

Objective: To determine the amounts of some of the naturally occuring auxins in plant tissue by bioassay.

Materials: Head of cabbage, oat seedlings, indoleacetic acid, diethylether, hexane, .01 M KH_2PO_4-NaOH buffer at pH 5 containing 2% sucrose, $MnSO_4$ solution (1 mg/l), distilled water, deep-freeze, vacuum pump, mortar and pestle, filter paper, large test tube (3 x 30 cm), razor blade, metric rule, dissecting microscope and occular micrometer.

Procedure:[3] Cabbage leaf tissue, which is known to contain indole-3-acetic acid and indoleacetonitrile, will be used as the test material. Remove about 6 grams of the etiolated inner leaf tissue and lyophilize by freezing in the deep-freeze and leaving overnight under a vacuum supplied by a good vacuum pump. When the tissue is thoroughly dry, pulverize it with a mortar and pestle, place the dry powder in a small flask, add 20 ml of diethylether, stopper with a cork

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[3] Ibid.

and extract with frequent shaking for 2 hours in the cold at about 4°C. Remove the solid plant material by passing the extract through filter paper. Concentrate the ether extract to about 5 or 6 ml by blowing air on the unstoppered flask (exercise caution). Stopper the flask and store in the cold.

Chromatography of auxins: The auxins may be separated by paper chromatography. Spot ½ of the ether extract on filter paper using a micropipette. Use a strip 2 cm wide and 25 cm long and place the spot about 5 cm from the bottom of the strip. Keep the spot as small as possible when placing the ether extract on the paper. This may be accomplished by placing the ether extract on the paper a drop at a time. Use a hair dryer or fan to aid the drying of the spot between drops. The chromatogram should be developed by the ascending technique using water-hexane as the developing solvent. Place 10 ml of water and 10 ml of hexane in a large test tube (3 x 30 cm.). Suspend the chromatogram in the tube so that the bottom end extends through the hexane layer and into the water layer about one cm. Allow the chromatogram to develop in the dark until the solvent front has moved about 20 cm. At this time remove the paper strips and air dry.

Bioassay of auxins: The quantity of auxins on the chromatogram may be determined by bioassay using the growth response of oat coleoptile as an indicator of the amount of auxins present. First cut the chromatogram into 1 cm sections

and place each section in a watch-glass. Add one ml of .01M KH_2PO_4-NaOH buffer at pH 5 containing 2% sucrose to the watch-glass. Place 10 four mm oat coleoptile sections in each solution. Preparation of the oat coleoptile sections is very important. Germinate oat seeds on washed sand at 30°C in the dark. Use the coleoptiles for the test when they have reached a length of 23-28 mm. Remove one 4 mm section from each coleoptile at a distance of 3 mm from the tip of the coleoptile. Remove the 4 mm sections with a small quillotine, made of razor blades, being careful to remove sections of consistant length. Before using the coleoptile sections, wash them for 2 hours with a solution of $MnSO_4$ (1 mg/l). Rinse the sections with distilled water before placing them in the auxins contained in the watch-glasses. After 24 hours, determine the length of each set of coleoptiles. The growth of coleoptiles in buffer-sucrose and in buffer-sucrose plus a known amount of auxin should also be determined. One microgram of indoleacetic acid (IAA) in the one ml of buffer-sucrose should be sufficient for this test. Determine the length of all the sections by using a wide-field microscope with an occular micrometer. Plot the results as mm of growth (in excess of the control) and as micrograms of auxin by using a bar graph corresponding to the position of the 1 cm paper sections removed from the chromatogram.

Exercise IV

Objective: To observe the effects of auxin, gibberellic acid and sucrose on the expansion growth of cells.

Materials: Cotton seed, sand, Shives nutrient solution, cork borer, 21 ten cm Petri dishes, analytical balance, 2,4-D, $KHCO_3$, gibberellic acid (K salt), light source and 2% sucrose.

Procedure:[4] Plant about 300 cotton seeds in sand wet with Shives nutrient solution at pH 5.5. Germinate for 8 days at 28-30°C under continuous fluorescent lighting. At the end of 8 days remove punches of cotyledon tissue with a number seven cork borer placing 20 punches in each of 21 ten cm Petri dishes after first determining the fresh weight of each set of 20 leaf punches. Add 10 ml of the following solutions to the Petri dishes: (a). To 7 dishes add 10 ml of Shives nutrient solution containing 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M 2,4-D. When preparing the 2,4-D solutions add equimolar $KHCO_3$ to convert the 2,4-D to the K salt. (b). To 7 dishes add 10 ml of Shives nutrient solution containing 10^{-4} M gibberellic acid (K salt) and 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M 2,4-D (K salt). (c). To 7 dishes add 10 ml of Shives nutrient solution containing 10^{-4} M gibberellic acid (K salt), 2% sucrose and 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M 2,4-D (K salt).

Place the dishes under fluorescent light at 30°C and leave for 24 hours. At the end of this time, blot the tissue dry and weigh. Plot the results as percentage increases in fresh weight. Evaluate the results and determine the effects of auxin, gibberellic acid and sucrose on the growth of plant cells.

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[4]Ibid.

Exercise V

Objective: To determine the light requirement for tobacco seed germination and to determine if this requirement may be replaced or nullified by other factors.

Materials: Turkish tobacco seed (should be less than two years old), gibberellic acid (K salt), filter paper, 21 ten cm Petri dishes and distilled water.

Procedure:[5] Place 100 Turkish tobacco seeds on 3 thicknesses of filter paper in each of 21 ten cm Petri dishes. Add 5 ml of distilled water to 15 dishes and 5 ml of 2×10^{-4} M gibberellic acid to the remaining 6 dishes. Keep the dishes in the dark for 24 hours at 15°C and then expose to the following conditions: Seeds in water. (a). Leave 3 dishes in the dark at 25°C for 4 days. (b). Expose 3 dishes to red light for 30 minutes and then leave for 4 days in the dark at 25°C. (c). Expose 3 dishes to far-red light for 30 minutes and then leave for 4 days in the dark at 25°C. (d). Expose 3 dishes to red light for 30 minutes, far-red for 30 minutes and leave in the dark for 4 days at 25°C. (e). Expose 3 dishes to red, far-red and then red again for 30 minutes each and leave in the dark at 25°C for 4 days. Seeds in 2×10^{-4} M gibberellic acid. (a). Leave 3 dishes for 4 days in the dark at 25°C. (b). Expose 3 dishes to far-red light for 30 minutes and then leave for 4 days in the dark at 25°C.

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[5]Ibid.

At the end of 5 days from the beginning of the experiment determine the number of seeds which have germinated in each dish. Evaluate the results.

Exercise VI

Objective: To determine what factors are involved in breaking the dormancy of buds.

Materials: Branches of lilac, pear, Japanese quince, pussy willow, oak or others.

Procedure:[6] Specimens of the above plants should be collected in the fall before freezing weather and again after being exposed to freezing temperatures for a few weeks. After the branches have been cut off and placed in water, cut off a segment an inch or two in length from the cut end while it is submerged in water. This removes air-plugged vessels which may prevent further entrance of water into the stems. For the forked branch of lilac, prepare a small opening in a window pane so that one branch may be exposed to the outside temperatures. With cardboard or other insulating material seal the opening about the exposed stem to prevent lowering of the temperature inside the window. The other stems may be kept in water at about room temperature. Observe the development of the buds and compare the results on those collected before and after the cold exposure. If an electric refrigerator is available the low-temperature exposure may be made artificially. Such exposure should

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[6]David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences, (New York, 1938), p. 324.

be made for a period of 2 weeks at near freezing temperatures.

Considerable work has been done on breaking dormancy of buds by means of chemical treatments. Some students may find this subject of interest. Information concerning this is available from the Boyce Thompson Laboratory for Botanical Research, Yonkers, New York.

Exercise VII

Objective: To determine what wavelengths of light are important in controlling the flower-inducing principle in cocklebur plants.

Materials: About 125 cocklebur seeds, 25 six-inch soil pots, razor blade or knife and a source of fluorescent light.

Procedure:[7] Plant about 5 cocklebur seeds in each of 25 six-inch soil pots. Before planting the seeds, remove the pointed tip of the seed so that the inner seedcoat has been ruptured. This can be accomplished by cutting off the extreme tip of the seed with a razor blade. Thin the plants to two plants per pot after the seedlings have emerged. Grow these plants on 17 hour day lengths. The day length may be lengthened during the evening hours by placing the pots under a fluorescent light. A timing mechanism, connected to the fluorescent light, is helpful if one is available. When the plants are 40 days old subject them to the following light conditions: (a). Continue to expose 4 pots

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[7]Eddie Basler, Personal Communication.

of plants to 17 hour day lengths. (b). Expose 4 pots of plants to 8 hours of day for 2 consecutive days. (c). Expose 4 pots of plants to 8 hours of day, 6 hours and 50 minutes of dark, 10 minutes of red light and 9 hours of dark during 2 consecutive 24 hour periods. (d). Expose 4 pots of plants to 8 hours of day, 6 hours and 50 minutes of dark, 10 minutes of far-red light and 9 hours of dark during 2 consecutive 24 hour periods.

Return all plants to 17 hour daylengths and count the number of flower buds at one month from the day of induction. Evaluate the resulting data in terms of length of exposure and wavelength of light involved with each treatment.

Section 2--Mineral Nutrition

Exercise I

Objective: To determine the percentage of total ash in various tissue.

Materials: Corn, woody twigs, wheat straw, potato tubers, alfalfa leaves, drying oven, desiccator, porcelain crucibles, tongs, analytical balance, Bunsen burner, concentrated nitric acid.

Procedure:[8] Determine the moisture content of the various samples by weighing the sample, heating in a drying oven at 100°C until constant weight is obtained and determining the final dry weight. Grind the dry samples with a mortar and pestle and place them in a desiccator. Heat a clean

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[8]Walter E. Loomis and Charles A. Shull, Methods in Plant Physiology, (New York, 1937), pp. 48-49.

porcelain curcible, one for each sample to be examined, at low red heat for ½ hour in a blue flame. Cool until the yellow color fades and then cool for an hour or more in a desiccator and weigh with quantitative precautions to ±0.1 mg. The crucibles must mot be touched, except with clean tongs, or exposed more than momentarily to moist or dusty air. Transfer the samples of the oven-dry material into crucibles, using from 0.5 to 10.0 gm and weighing again to 0.1 mg. Heat the crucibles in a Bunsen flame at low red heat for 3 hours or until free of black or gray color. If the carbon color persists, cool, moisten the ash with a drop or two of concentrated nitric acid, and reheat. Cool for a minute in the air and then in the desiccator and reweigh. Calculate the percentage of total ash in the oven-dry material.

Exercise II

Objective: To demonstrate the presence of various elements in plant ash.

Materials: Ash samples, concentrated hydrochloric acid, distilled water, barium chloride, ammonium hydroxide, ammonium oxalate, dibasic sodium phosphate, potassium thiocyanate, silver nitrate, concentrated nitric acid, molybdate solution (see appendix), distilled water and filter paper.

Procedure:[9] Dissolve about 0.2 gm of ash in 10 ml of warm 1 / 4HCl (one part concentrated HCl and four parts distilled water by volume) and make to a volume of 100 ml with dis-

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[9] Ibid. pp. 49-50.

tilled water. Test for various elements as follows:

Sulfur: Add a few drops of $BaCl_2$ to a 10 ml portion of the filtered ash solution. A white very finely crystalline precipitate is $BaSO_4$.

Calcium: Take a second portion of 20 ml and make slightly alkaline with 1 ∕ 1 NH_4OH. Filter and add a few drops of a saturated ammonium oxalate solution. A white precipitate is calcium oxalate. Save this.

Magnesium: Add an excess of ammonium oxalate to the calcium test solution to precipitate all calcium. Filter and evaporate the filtrate to a volume of 10 ml. Add 1.0 ml saturated Na_2HPO_4 solution, cool and allow to stand. Crystals are NH_4MgPO_4. Crystallization may be hastened by rubbing the inside of the vessel with a glass rod.

Iron: To a few drops of the ash solution on a white plate, add a drop of potassium thiocyanate solution. A red color indicates $\left[Fe(CNS)_6\right]^{\equiv}$.

Sodium: Test a loopful of the solution, concentrated if necessary, in a clear flame on a sodium-free wire. A yellow flame color indicates sodium.

Chlorine and phosphorus: Dissolve a second sample of ≤ 0.1 gm of ash in 1 ∕ 9 HNO_3 and divide into two unequal portions. To the smaller add a few drops of silver nitrate solution and observe for a heavy white percipitate of AgCl as an indication of the presence of chlorine. To the larger portion add molybdate solution, heat on a steam bath for a few minutes and cool. A yellow precipitate is ammonium-

phospho-molybdate.

Other minor elements can sometimes be detected in plant ash. For tests for these elements, refer to a chemistry manual.

Section 3--Water Relations

Exercise I

Objective: To determine root pressure and xylem exudation.

Materials: Woody-stemmed potted plant (geranium, tomato or sunflower), knife, rubber tubing, soft wire, mercury, glass T-tube, glass bottle, two-holed rubber stopper, capillary tubing, glass elbow and hose clamp.

Procedure:[10] Root Pressure. Thoroughly moisten the soil in the pot. Using a sharp knife, cut off the stem of the plant 1-2 inches above the soil. Attach a glass T-tube to the stump of the plant with rubber tubing and wire. Fill the tube with water. Arrange a heavy-walled glass bottle with a tightly fitting two-hole rubber stopper and with capillary tubing in one hole and a bent glass elbow in the other. Carefully pour into the bottle about ½ inch of mercury, so that the lower end of the capillary tubing is well covered. Now attach the glass elbow to the horizontal arm of the T-tube with rubber tubing and wire in position. Pour water into the system through the top of the T-tube, allowing it to run over into the bottle to fill the system completely.

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[10]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology, (San Francisco, 1956), pp. 66-67.

Be careful to avoild trapping air bubbles in the bottle or in the glass or rubber tubing. Allow the tubing at the top of the T-tube to remain open until the initial period of water absorption by the plant is over; then fill up the tube again and clamp it tightly closed. The mercury in the capillary will rise and can be followed for days. Make all measurements from a marked place on the capillary tubing. To convert the measurements of column height into atmospheres of pressure, the following figures, which will be approximately correct for normal laboratory conditions, may be used as a basis for calculation: Mercury column (specific gravity of mercury = 13.546): 76.0 cm of mercury equals 1 atm pressure.

Before dismantling the experiment, and while the level of the liquid in the capillary is still high, pour 50 ml of saturated NaCl solution on the soil. Observe the level of the liquid in the capillary.

Xylem Exudation: Select a plant similar to that used in the previous experiment. Cut off the top of the plant as before and, using a piece of rubber tubing, attach to the stump one glass U-tube with the end of the tube hanging over a 100 ml graduated cylinder. Cover the mouth of the cylinder with aluminum foil to prevent evaporation. Keep the soil moist. Collect the xylem exudate for the next 48 hours. Measure the volume collected, discard and repeat for a second period. What conclusions can be drawn concerning water in the vascular tissue of a plant?

Exercise II

Objective: To determine the osmotic pressure of cell sap.

Materials: Sucrose, test tubes, scalpel, forceps, microscope and leaves of Rhoeo discolor.

Procedure:[11] Fill each of 8 test tubes about 1/3 full with the following series of sucrose solutions: 0.28, 0.26, 0.24, 0.22, 0.20, 0.18, 0.16 and 0.14 M. With a scalpel and a pair of forceps carefully peel off small strips of the lower epidermis of leaves of Rhoeo discolor. Examine a strip of the epidermal tissue to verify that it consists of intact cells containing the red-colored anthocyanin pigment dissolved in the vacuolar sap. Drop a strip into each tube, at about 4-minute intervals between successive tubes. Exactly 30 minutes after immersion of each strip, examine the cells under the microscope for plasmolysis, mounting the strip in the same solution in which it has been immersed. Count cells showing any plasmolysis as plasmolyzed. Examine about 25 cells. Record in tabular form the number of cells plasmolyzed and unplasmolyzed in the various solutions, and plot the percentage of cells plasmolyzed against the molar concentration of sucrose. The solution in which half of the cells are plasmolyzed is considered to have the same osmotic pressure as the cell sap. Calculate the osmotic pressure of the epidermal cells by the following formula:

$$\text{osmotic pressure} = \frac{22.4\ MT}{273}$$

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[11] Ibid. p. 62.

in which M = concentration of external solution at incipient plasmolysis (moles/liter), and T = absolute temperature ($0^{o}C = 273^{o}$ T on the absolute-temperature scale; add to this value the temperature of the laboratory in degrees centigrade). If this formula is to be used for electrolyte solutions, a correcting factor for ionization must be introduced. In all cases, application of this formula is limited to rather dilute solutions--less than about 0.5 M.

Section 4--Cellular Metabolism

Exercise I

Objective: To study the colloidal nature of protoplasm.

Materials: Head of cabbage, blender, cheesecloth, filter paper, distilled water, test tubes, 1% glucose solution, microscope, slide, cover slip, Bunsen burner, ice, 95% ethyl alcohol, ammonium sulfate, dialyzing tube, 1% NaCl solution, 5 ml pipette, thread, glass rod and large beaker.

Procedure:[12] Preparation of Plant Sap. Grate into shreds a quarter of a head of cabbage, grind up thoroughly in a blender, squeeze the pulp through cheesecloth, and collect the expressed sap. Filter the cabbage-leaf juice, and make up to 40 ml with distilled water. This juice, representing the expressed vacuolar and protoplasmic contents of the cells, contains many plant substances in solution or colloidal dispersion. Much of the colloidal material is derived from proteinaceous components of the protoplasm. The following experiments carried out on the cabbage-leaf juice illustrate

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[12]Ibid., pp. 78-79.

certain aspects of the colloidal behavior of protoplasm.

A. Tyndall Effect. Pour 10 ml of the cabbage juice into a test tube, and in a darkened room pass a narrow beam of light through the liquid. Similarly pass the light through a crystalloidal solution, such as a 1% solution of glucose, and notice the difference. Save the cabbage juice for a later part of this experiment.

B. Brownian Movement. Place a drop of the juice on a slide, cover with a glass cover slip, and examine with the high power of the microscope. Note the vibrating motion of the small visible particles.

C. Precipitation of Hydrophilic Sols. Pour 5 ml of the cabbage juice into each of 6 test tubes. Number the tubes from 1 to 6, and subject to the appropriate treatment indicated. (1). Control, keep at room temperature. (2). Heat to boiling. (3). Cool to freezing, then thaw. (4). Add 1.5 grams of sucrose to the juice, then cool to freezing, then thaw. (5). Add 5 ml of 95% ethyl alcohol. (6). Saturate with solid ammonium sulfate $[(NH_4)_2SO_4]$, shaking constantly. After 2 hours observe each tube for indications of precipitation.

D. Dialysis of a Colloidal Suspension. Place about 10 ml of cabbage juice in a dialyzing tube. Add 0.5 ml of 1% NaCl solution. Tie the sack closed with a linen thread, and fasten the thread to a glass rod. Place the rod across the top of a beaker containing distilled water, allowing the sack to be completely immersed. Immediately withdraw

5 ml of distilled water from the beaker, acidify with a few drops of concentrated nitric acid, and add a few drops of 10% silver nitrate. A white precipitate of silver chloride is produced if chloride is present. After 2 hours retest the dialyzing solution for the presence of chloride, using the silver nitrate test.

Exercise II

Objective: To determine sugar-starch interconversions in leaves.

Materials: Variegated geranium leaf, iris leaf, etiolated corn leaves, bean seeds, 95% ethyl alcohol, I_2KI solution, 0.5 M solutions of sucrose, glucose and fructose, distilled water, Petri dish, Bunsen burner, three 50 ml beakers, sand, 6-inch soil pot, wooden applicator sticks, drafting tape, glass jet and rubber tubing.

Procedure:[13] A. Starch Storage in Leaves. Obtain, from plants that have been in bright light for several hours, 1 leaf of variegated geranium and 1 of iris. Immerse the leaves in 95% ethyl alcohol on a steam bath for 20 minutes then wash them in hot water, and immerse them in I_2KI solution in a Petri dish for a few minutes. Rinse off the iodine solution with water, and spread out. A dark purple coloration indicates the presence of starch in the leaf.

B. Interconversion of Sugars. Put into 50 ml beakers each of the following: 0.5 M solutions of sucrose, glucose and fructose, and distilled water. Pick 9 etiolated corn leaves, and place the basal ends of 2 of them in each of the

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[13] Ibid., p. 91.

solutions. Mark each pair in such a way that they can be identified. Keeping the basal ends in the solutions, cut off about 1 cm of the base of each. Place the beakers on a shelf. Test the odd leaf for starch as in part A above. After 48 hours, test the leaves in the solutions for starch.

C. Translocation of Carbohydrates. Plant 5 soaked bean seeds in a soil pot filled with sand. Water thoroughly, and place in a well lighted window. After the plants have developed, remove all but the 2 healthiest, and allow these to grow for about 4 weeks, or until the first pair of trifoliate leaves have expanded. Immediately before removing the plants to the laboratory, give them continuous bright light for several hours. In the laboratory, test 1 of the primary leaves for starch according to the directions given in part A above. If the test is strongly positive, proceed with the experiment as follows.

To prevent interference with xylem conduction, support the petiole of each of the trifoliate leaves by taping an applicator stick under its length, securing the ends of the stick to the ends of the petiole with drafting tape. Kill a $\frac{1}{2}$ inch segment in the middle of 1 petiole of each plant with a fine jet of steam projected through a glass tube drawn to a fine point. Take care to avoid injury to the stem or leaves.

After steaming 1 petiole of each plant, place both plants in complete darkness at 25°C. Two days later remove one leaflet from each of the treated and untreated tri-

foliate leaves, marking the leaflets from the untreated leaves with a small tear so that they may be identified later. Test these leaves for starch, using the I_2KI test performed earlier. Repeat the test for starch on another set of leaflets on the seventh day after steaming. Compare the amounts of starch present in the treated and untreated leaves.

Exercise III

Objective: To determine the composition of the cell wall.

Materials: Concentrated sulfuric acid, concentrated hydrochloric acid, sodium hydroxide, Benedicts reagent, Bial's reagent (0.2% solution of orcinol in concentrated HCl), 2 N ammonia, 75% ethyl alcohol, alcoholic phloroglucin solution, 4% solution of benzidine in glacial acetic acid, 0.5 M calcium chloride, glucose, fructose, sucrose, zylose, wood shavings, potassium iodide solution, potassium permanganate, apple, Buchner filter, filter paper, test tubes, surgical cotton and Bunsen burner.

Procedure:[14] A. The Structural Carbohydrate: Cellulose. To 5 ml of concentrated H_2SO_4 in a small flask add small pieces of surgical cotton (about 0.3 g). When the cotton is dissolved, pour the solution slowly and cautiously into 15 ml of water, taking care to cool the flask under the tap. Take 10 ml of the cooled solution, add a drop of phenolphthalein, and neutralize with 10 N NaOH until a faint pink color results. Heat the remaining 10 ml on the steam bath for 10 minutes, cool, and neutralize. Make the following

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[14] Ibid., p. 97-98.

tests on portions of each solution after determining positive tests with solutions of known sugars (e.g., glucose, fructose, sucrose and zylose): (1). Benedict's reagent for reducing sugars. (2). Seliwanoff's reaction for ketoses. To 1 ml of solution add 1 ml of 25% HCl and a pinch of resorcinol. On warming, a deep rose-red coloration indicates a ketose. (3). Bial's test for pentoses.

Stain a thin section from a young tomato stem on a slide in I_2KI solution for 1 minute. Drain off the iodine solution, add a drop of 75% H_2SO_4. Apply cover slip and observe under the microscope. Structures of cellulose become blue and swollen.

B. Lignin. (1). Stain a thin cross-section of a young tomato stem on a slide with a drop of alcoholic phloroglucin solution. Allow the solution to evaporate. Add a drop of concentrated HCl, then a cover slip. Examine. Lignin stains cherry-red. (2). The permanganate test may be used to distinguish between the lignins of hard and soft woods. Soak wood shavings in 1% $KMNO_4$ solution for 15 minutes. Rinse several times in distilled water, and then soak in 25% HCl for 5 minutes. Wash well with distilled water, and add a few drops of 2 N ammonia. Hard woods show a deep red color; soft woods appear brown. These chemical differences are not well understood.

C. Pectin. Obtain the freshly pressed juice from a chopped apple. Add 3 times the volume of 75% alcohol to the clear solution, allow to warm on a steam bath for $\frac{1}{2}$ hour,

and filter the precipitated pectin in a Buchner filter. Wash the precipitate with 75% alcohol, scrape if from the filter paper, and dissolve it in 10 ml of distilled water.

To 1 ml of the solution add (in the hood) 2 ml of 4% solution of benzidine in glacial acetic acid, heat to boiling and cool rapidly. A bright red color is characteristic of pentoses, probably represented here by arabinose, present in the form of nonpectic arabans.

Divide the remaining pectin solution into two 4 ml portions in test tubes. To one add 2 ml of 2 N NaOH, warm gently, then cool. To the other add 2 ml of distilled water, and allow to stand for 10 minutes. Test 3 ml portions of each solution as follows: (1). Acidify with 0.5 ml of concentrated HCl. Pectic acid will form a gelatinous precipitate. (2). Add 0.5 ml of 0.5 M $CaCl_2$. The precipitate formed is calcium pectate.

Section 5--Photosynthesis

Exercise I

Objective: To extract the various chloroplast pigments in green leaves.

Materials: Spinach leaves, 80% acetone, petroleum ether, distilled water, methyl alcohol, ethyl ether, 30% methyl alcoholic potassium hydroxide, Buchner funnel and separatory funnel.

Procedure:[15] Mix 2.5 grams of dried, ground spinach leaves

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[15]Ibid., p. 136.

with 40 ml of 80% acetone. When the acetone is colored deep green, filter on a Buchner funnel. The extract contains the chloroplast pigments.

Put 50 ml of petroleum ether into a separatory funnel, and add the acetone solution of the chloroplast pigments. Gently rotate the funnel. Add 70 ml of distilled water by allowing the water to flow down the side of the funnel. Rotate the funnel until the upper layer is quite green. Permit the layers to separate, and then draw off the lower acetone-water layer and discard.

Wash the petroleum ether solution by adding 50 ml of distilled water. Gently rotate the funnel and drain off the water. Do this twice more.

To the clean petroleum ether solution add 50 ml of 92% methyl alcohol, mix by rotating, and then draw off the lower methyl alcohol solution into one beaker and the upper petroleum ether solution into another. Methyl alcohol is poisonous. Avoid the fumes as much as possible. Chlorophyll a and carotene are now in the petroleum ether solution, chlorophyll b and zanthophyll are in the methly alcohol.

Put 50 ml of the methyl alcohol solution containing chlorophyll b and zanthophyll into the separatory funnel, add 50 ml of ethyl ether, and mix by rotating. Now add distilled water, 5 ml at a time down the side of the funnel, rotating after each addition, until two layers of liquid appear. This usually requires 25 ml or more. Discard the lower methyl alcohol layer.

Put 30 ml of the ethyl ether solution into a large test tube and 30 ml of the petroleum ether solution into a second tube. Carefully pour 15 ml of fresh 30% methyl alcoholic potassium hydroxide solution down the wall of each container, shake, and then observe for 10 minutes. Add 30 ml of distilled water to each tube, shake, and permit the two layers to separate.

Exercise II

Objective: To separate the chloroplast pigments by adsorption chromatography.

Materials: Petroleum ether solution of the chloroplast pigments, magnesium oxide powder, petroleum ether, benzene, Pyrex glass chromatographic tube, ring stand, clamp, glass funnel, filter flask and vacuum source.

Procedure:[16] Prepare a petroleum ether solution of the chloroplast pigments using the procedure described in the previous exercise. Pack, with magnesium oxide (heavy) powder, a Pyrex glass chromatographic tube of convenient size (about 3 x 20 cm) fitted with a fritted glass disk. Dry packing of the column is most easily achieved in the following manner. Support the tube vertically with the ring stand and clamp. Using a large-bore glass funnel, pour the magnesia into the tube slowly, and at the same time rap the side of the tube sharply with a wooden stick to settle the powder. After adding a few cm of powder,

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[16] Ibid., p. 138.

pack it tightly with a packing rod of a diameter that exactly fits the tube. Repeat the procedure until the tube is filled to within 5 cm of the top. Uniform packing is essential.

Mount the chromatographic tube on a heavy-walled filter flask, and apply gentle suction to the side arm. Using a glass stirring rod, gently pour the previously prepared petroleum ether solution down the side of the tube, wetting the entire surface of the magnesia. Pour in enough pigment solution to nearly fill the tube. The suction draws the solution down through the magnesium oxide powder. Because of their differential adsorptive properties, the different pigments in solution are adsorbed to the particles of the column more or less tightly, and each pigment forms a distinct color band as the solution is drawn through.

When nearly all of the pigment solution has passed into the magnesium oxide, gently add about 50 ml of a 1:1 mixture of petroleum ether and benzene to "develop"--that is, to spread out the bands. In order, from the top of the column, the following bands will be distinguishable: The chlorophylls, with chlorophyll b forming the yellow-green top layer and chlorophyll a the lower dark blue-green band; the zanthophylls; and the carotenes, with beta-carotene the darker orange-yellow zone and alpha-carotene the lowest band of brighter yellow.

PART III

ECOLOGICAL FACTORS AFFECTING PLANTS

Introduction

The word ecology is derived from a Greek word meaning "house" and refers to the study of plants and animals and their relation to their natural homes or environments.

This part is confined to plants as they are more accessible, to elementary ecology students, than animals and essentially plants are the basis of all life on earth. Animals, including man, only use the results of the works of plants but are an important factor in any community.

Ecology, in the strict sense, is probably not introduced to the high school biology student although it is approached from several angles. It perhaps cannot be defined as a separate branch of botany but is more a means of approach to a large part of detailed botanical study. Taxonomy is involved as different species with which the ecologist works must be recognized. A knowledge of their structure is essential so morphology is introduced. Sometimes the minute anatomy of the plant is a basis for understanding so cytology must be studied. Lastly, the field of physiology is involved. This phase of botany is probably

most closely related to ecology as here the ecologist attempts to understand the reasons some plants flourish in particular situations while others do not. This leads to a study of soil and climate and their relation to various species. This in turn leads to a study of the physical and chemical relations of the plant and its habitat.

Ecology should be studied primarily in the field, however, substantial background in taxonomy, morphology and physiology is necessary for much of the field work. Such field work includes vegetation studies by quadrats and transects, plant succession, units of vegetation, migration and competition. Most high school students do not have the background and the weather conditions during the school year are limiting factors, therefore, this part includes exercises on various aspects of ecology which can be conducted in the laboratory. These are relatively short and simple but will acquaint the student with certain ecological principles which he may later encounter in the field.

Section 1--Soil Relations

Exercise I

Objective: To demonstrate various mineral deficiencies in growing plants.

Materials: Magnesium sulfate, calcium nitrate, potassium dihydrogen phosphate, ferric tartrate, manganese chloride, sodium sulfate, magnesium chloride (anhydrous), sodium nitrate, calcium chloride, sodium dihydrogen phosphate (anhydrous),

potassium chloride, distilled water, eleven bottles (100 cc or larger), analytical balance, graduated cylinder, seven glazed or paraffined soil pots (4 inch), pure silica or quartz sand, tomato seed and labels.

Procedure:[1] Make up the following solutions which represent the essential elements for a complete cultural medium. (1). Magnesium sulfate, $MgSO_4$ (anhydrous), with distilled water added to make volume of 100 cc ...12 g. (2). Calcium nitrate, $Ca(NO_3)_2$, with distilled water added to make volume of 100 cc ...16.4 g. (3). Potassium dihydrogen phosphate, KH_2PO_4, with distilled water added to make volume of 100 cc ...13.6 g. (4). Ferric tartrate, 0.2 per cent ...100 cc. (5). Manganese chloride, 0.2 per cent ...100 cc.

Solutions deficient in each of the mineral elements are made from the following substitutes. (1). Sodium sulfate (Na_2SO_4) with distilled water added to make volume of 100 cc ...14.2 g. (2). Magnesium chloride (anhydrous) $MgCl_2$, with distilled water added to make volume of 100 cc ... 9.5 g. (3). Sodium nitrate ($NaNO_3$) with distilled water added to make volume of 100 cc ...8.5 g. (4). Calcium chloride ($CaCl_2$) with distilled water added to make volume of 100 cc ...11.1 g. (5). Sodium dihydrogen phosphate (anhydrous) NaH_2PO_4 with distilled water added to make volume of 100 cc ...12 g. (6). Potassium chloride (KCl) with distilled water added to make volume up to 100 cc ...7.4 g.

If the above stock chemicals are not anhydrous, that is,

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[1]David F. Miller and Glenn W. Blaydes, Method and Materials for Teaching Biological Sciences, (New York and London, 1938), pp. 318-319.

if the compounds contain water of hydration, the weights must be modified as follows: Magnesium sulfate, 24.6 g; magnesium chloride, 19.3 g; sodium dihydrogen phosphate, 13.8 g. In all solutions be certain to add distilled water to the chemical making the volume up to 100 cc.

Carefully clean the soil pots and rinse with distilled water. Fill each with sand to within about 1 inch of the top. To each pot transplant six seedlings, which have been grown from seeds placed in moist soil or peat, just at the time they show the first tube leaves developing above the cotyledons. After they are well established, thin to three seedlings. To make up a complete mineral nutrient use the following amounts of the various solutions given above: $MgSO_4$ - 7.2 cc, $Ca(NO_3)_2$ - 4.8 cc, KH_2PO_4 - 7.2 cc, ferric tartrate, 0.2% - 2 cc and manganese chloride, 0.2% - 2 cc.

Mix the above and add enough distilled water to make 1 liter. This is the complete cultural solution. To make deficiency solutions modify as follows: Use 7.2 cc of Na_2SO_4 to replace $MgSO_4$, for deficiency of magnesium. Use 7.2 cc of $MgCl_2$ to replace $MgSO_4$, for deficiency of sulfate. Use 9.6 cc of $NaNO_3$ to replace $Ca(NO_3)_2$, for deficiency of calcium. Use 4.8 cc of $CaCl_2$ to replace $Ca(NO_3)_2$, for deficiency of nitrate. Use 7.2 cc of NaH_2PO_4 to replace KH_2PO_4, for deficiency of potassium. Use 7.2 cc of KCl to replace KH_2PO_4, for deficiency of phosphorus.

Label each of the seven pots to show what is to be

received. Place the pots in a well-lighted situation at room temperature. At the beginning water each thoroughly with its proper mineral nutrient solution using equal volumes on all seven. Application of these solutions should be made every 2 or 3 weeks. During intervening periods apply distilled water to each in equal volume. Watch the plants from day to day and take note of differences such as color, size and development of roots, stems and leaves. After 30 to 40 days take final notes. Weight of tops and root systems may be obtained if desired. Record all data and draw conclusions.

Exercise II

Objective: To determine pH by the colorimetric method.

Materials: Pipette, porcelain dish, various indicators, color chart, liquid color standards and various soil samples. Vegetable juices may give better results.

Procedure:[2] Pipette 5 ml of tap water into a clean porcelain dish and add 2 drops of universal indicator. Compare the color with the color chart for the universal indicator and determine the pH of the tap water as closely as possible. From the color chart select an indicator dye such that the pH just determined falls in the middle of the range of color change.

If color charts for the individual indicators are

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[2]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology, (San Francisco, 1956), p. 49.

available, perform the pH determination as follows: To 5 ml of tap water add 2 drops of the selected indicator and compare with the color chart for the indicator.

If liquid color standards are available, refine the pH measurement as follows: Using a test tube of the same size as those holding the color standards, add tap water to the same height as that in the color standards. Now add 10 drops of indicator, shake and compare the color with that of the standards.

By the above methods determine the pH of distilled water, various soil samples and plant juices. When testing soil samples, sufficient time should be allowed for most of the suspended material to settle out.

Exercise III

Objective: To determine the water content of soils.

Materials: Sunflower seedling, samples of various soil types--sand, clay, loam and peat, can with lid, filter paper, wire screen, balance, cotton, cork, oven and humidity chamber.

Procedure:[3] Part A. Water Content of Soils as a Percentage of Dry Weight. Examine the soil types. Feel the texture when dry; then moisten a few grams of each and note the texture.

Determine the water content of each soil by the following procedure: Weigh a can plus filter paper when dry.

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[3]Ibid., pp. 74-75.

Place about 4 cm of soil in the can and tap gently several times to settle. Allow the can to stand in shallow water under cover for about 2 to 5 days. Place the can on a wire screen and allow it to drain for 48 hours; then weigh. Dry the cans of soil at 105°C for 48 hours or more. Cool and weigh; then calculate the grams of water per gram of dry soil.

Part B. The Permanent Wilting Percentage of a Soil. Weigh an empty pound-size coffee can, without its lid, to the nearest 0.01 g. Then fill the can to within ½ inch of the top with moistened sandy loam. Test the lid provided to see that it fits snugly. The lid will have been prepared with a central perforation about ½ inch in diameter and a smaller hole near the edge.

Transplant the sunflower seedling to the center of the prepared can of soil with the seedling shoot projecting through the central hole in the lid. Protect the stem from the sharp edge of the hole with a wad of nonabsorbent cotton, which will also prevent evaporation via the central hole. Add a small additional amount of water, stopper the smaller hole with a cork and place the can in a lighted window. Add small amounts of water through the second hole until the plant is well established and has produced four pairs of expanded leaves.

At this stage of development, stop adding further water. Observe the plant daily for signs of wilting until it appears that permanent wilting has occurred. Now transfer the can to a humid chamber for overnight. If the plant

does not recover, it is considered to be permanently wilted.

When this stage has been reached, remove the lid of the can, together with the plant, shaking off as much soil from the roots as possible. Weigh the can containing the moist soil, dry the soil in the can at 105°C for 48 hours, cool and reweigh. Calculate the grams of water per gram of dry soil and express as the permanent wilting percentage.

Exercise IV

Objective: To determine the effects of soil type on plant growth.

Materials: Various soil types--heavy clay, light sandy loam and sandy loam (or others), 3 or more small pots and bean seeds.

Procedure:[4] Examine the different types of soil. Note the differences in their color and texture. Test the pH of each by the procedure outlined in exercise II.

Select 3 small pots and fill each with a measured quantity of the different soils. Plant the same number of bean seeds in each of the pots and place them in a window where they will be given adequate light. Water as needed.

To test the reaction of different species to these soils use the procedure outlined above and plant wheat, radish or other rapidly growing annuals.

Examine the plants once a week. At the end of a four week period, remove the plants from the pots and measure the

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[4]J.T. Curtis, Plant Ecology Work Book, (Minneapolis, 1950), p. 4.

difference in their relative growth using such characters as average plant height, weight, number of nodes etc, etc. Determine which soil produced the best plants. If other species of plants were also tested, determine the reaction of different species to similar soils in which the beans were growing. If possible, examine a map showing the distribution of the main soil groups throughout the world and determine the correlation in the temperate region between population concentration and distribution of soils.

Section 2--Reaction to Environment

Exercise I

Objective: To show phototropic and geotropic response in plants.

Materials: Oat seeds, corn seeds, indoleacetic acid, Petri dishes, moist chamber, wire screen holder, tin can, razor blade, metric rule, lanolin paste, protractor and wax pencil.

Procedure:[5] Phototropism. Remove the husks from 20 oat seeds. Soak in distilled water for 24 hours at 25°C. Prepare a moist chamber by lining the sides of a 600 ml Griffin beaker with thoroughly moistened paper toweling. Omit the paper toweling on the bottom of the beaker. One side of the beaker should be completely free of toweling for about 3 inches. Using the soaked oat seeds, select 12 seeds of about equal size, arrange them in the wire screen holder with the grooved side of the seed down and the coleoptile

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[5]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology, (San Francisco, 1956), pp. 22-23.

pointing vertically upward. Fill the beaker with tap water to a level just below the wire screen. Cover the beaker with the top of a Petri dish and then cover the entire assembly with a large inverted tin can to give complete darkness. Place the apparatus in the dark at 25°C. Constant temperature for the next 48 hours is essential.

After 48 hours, carry out the following procedure in a darkroom illuminated only by dim red light. Designate three sets of three seedlings each for the following test, using every third seedling in the row for any set. Arrange the coleoptiles so that they are as nearly vertical as possible.

Group 1. With a razor blade, remove the apical 3 mm of the coleoptile. Place a small dab of plain lanolin paste on each cut surface.

Group 2. With a razor blade, remove the apical 3 mm of the coleoptile. Place a small dab of lanolin paste containing 0.1 percent indoleacetic acid (1,000 ppm) on each cut surface. See that the lanolin paste is applied uniformly to the entire cut surface.

Group 3. Leave the plants intact as controls.

Place the beaker in a position where the coleoptiles will receive, through the side of the beaker, direct, unilateral white light, perpendicular to the coleoptiles, for the next 2 hours. At the end of 2 hours, remove the holder with the seedlings, and measure the angle of curvature of all plants with a protractor, using the tangent to the curve

as the point of measurement from the vertical. Average the angles of curvature for the three sets and enter the averages in a report.

Geotropism. Select 6 corn seeds, soaked overnight in distilled water and arrange them across the center of a Petri dish with the embryos down and 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 each dish with a wax pencil. Remove the apparatus to the darkroom at 25°C.

After 48 hours, observe the seeds. Carefully excise, with a razor blade, the terminal 2 mm of all of the roots in one dish. 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 then intact. Rotate 90° to make the roots horizontal. Return the dishes to the dark at 25°C. At the end of 2 hours, observe the two sets of roots and record the results in the form of a diagram.

Exercise II

Objective: To determine the effect of environmental factors on the rate of transpration.

Materials: Leafy branch, potometer, knife and Vaseline.

Procedure:[6] Mount a leafy branch in the potometer as follows: Insert the end of the branch through the hole in a rubber stopper so that it fits snugly but is not compressed. About 2-3 inches of the stem should protrude below the stopper. Holding the cut end of the branch under water, cut off about 1 inch with a knife. Leave the stem immersed in water.

With the stopcock of the potometer open and a finger over the hole near the end of the capillary, fill the potometer with water through the reservoir. Close the stopcock. Holding the potometer securely under the well, insert the stopper. Make certain that no air bubbles are trapped in the well. Seal around the stopper and shoot with Vaseline if necessary.

Now allow the shoot to transpire with the end of the capillary exposed to the air. After a bubble of air has entered the capillary, immerse it in the beaker of water. When the bubble proceeds beyond the ruler, drive it back by partially opening the stopcock.

Determine the rate of transpiration--the distance moved by the bubble in the capillary per unit of time--in the laboratory on top of the desk, in the laboratory in front of a fan, and in the path of the light from two photoflood bulbs. For each condition continue measuring until you have three successive measurements that show close agreement.

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[6]Ibid., pp. 70-71.

After the last determination, remove all but 12 leaves (leave the largest 12 on the plant), and determine the rate in front of the photofloods. Now carefully cover the upper surface of each leaf with Vaseline and determine the rate. Finally, cover the lower surfaces with Vaseline and determine the rate.

Exercise III

Objective: To determine the effects of light intensity and temperature upon the rate of photosynthesis.

Materials: Elodea plant, 1000-watt lamp, four 1000-ml beakers, four thermometers, ice and atomizer.

Procedure:[7] Light Intensity. Carefully attach a vigorous shoot of Elodea to a glass rod with the cut end of the plant stem up. Immerse in tap water and observe the small bubbles given off from the cut stem when the plant is exposed to light. Adjust the size of the bubbles by varying the slant of the cut stem surface until they can be counted conveniently. The number of bubbles emitted in unit time is taken as an approximate measure of photosynthetic activity.

Place a plant at such a distance (2 or 3 m) from a strong artificial light set up without a reflector that bubbles are given off slowly. Count the number per minute. Reduce the distance between the plant and light by half and again record the number of bubbles per minute. One or two

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[7]Walter E. Loomis and Charles A. Shull, Methods in Plant Physiology, (New York and London, 1937), pp. 110-112.

minutes will be necessary for the plant to become adjusted to the stronger light, but heating should be carefully watched. Reduce the distance again to one-fourth and one-eighth of the original and record. Plot number of bubbles against relative light intensity, remembering that light intensity varies inversely as the square of the distance. The light intensity at the maximum distance may be measured, or it may be taken as unity, and the relative intensity of the shorter distances calculated.

The Effect of Temperature. Fill four 1000-ml beakers with water and adjust the temperature of the water with finely chipped ice and warm water to give the four temperatures 10, 20, 30 and 40°C. Hold the beakers at these temperatures and force laboratory air through them vigorously with an atomizer to saturate the water with air. Choose a vigorous sprig of Elodea, adjusted to bubble satisfactorily, and determine the bubbles evolved per minute when the plant is held for 2 to 5 minutes in each of the beakers. Avoid disturbing the plant in moving and provide an adequate and uniform light source. The plant should be held at each temperature for a short time before the readings are taken.

Temperature control experiments with land plants are possible, however, they are more difficult and require equipment not readily available in the high school.

PART IV

PLANT PATHOLOGY

Introduction

Plant pathology as defined by Heald[1] is that phase of botany which deals with the diseases of plants. These diseases are any alteration of the state of a plant or of some of its organs or parts, interrupting or disturbing the performance of vital functions, or as any departure from the state of health presenting marked symptoms or injurous effects.

This phase of plant science has evolved from the early recordings of various rusting and mildewing to the complex science of today which ranges from physiological disorders to virus infections.

The seriousness and importance of plant pathology is easily recognized when we but think of the destructiveness of such diseases as the Irish potato blight which devastated million of acres in Europe, the United States and Canada, chestnut blight which has wiped out practically all the chestnut trees in the United States and a more

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[1]Frederick D. Heald, Introduction to Plant Pathology (New York and London, 1943), p. 1.

recent example; Dutch elm disease which was introduced into the United States from Europe and is currently a threat to the American elms. Countless numbers of such examples could be listed to emphasis the importance of plant pathology and research into methods of controlling the ever present scourge of plant disease. Only through continued intensive research by trained plant pathologists will this threat be kept under control.

The pathologists of the future are the students of today. They must be introduced to pathology and its possibilities and encouraged to enter this field of botany.

No attempt is made in this paper to build up the students background in pathology. It is merely to introduce this science to him, developing in him an awareness of plant diseases and to interest and encourage him in this field. In these exercises emphasis is placed on methods of isolation, innoculation and the demonstration of pathogenicity of various organisms. As is true of any experimental project a review of the literature is a prerequisite which is essential, not only to prepare the student for the experiment, but also to develop in him an appreciation of the subject. For this reason a review of all available literature is suggested before the exercises are attempted.

The first two exercises in this part should be completed before the student attempts demonstrating the pathogenicity of a particular organism. It is in these exercises that techniques, which are referred to later, are developed.

Elasticity is important in these exercises as the pathogens with which a student works depends upon many factors such as locality, season of the year and amount of time which can be devoted to the project. A great variety of organisms in each category may be used and of course the procedure may vary somewhat depending upon the pathogen. A particular situation is indicated in these exercises merely to serve as a guide.

It is the opinion of the author that through projects such as these the student will realize the importance of plant pathology to our national economy, consider its possibilities and perhaps become interested enough to at least consider this phase of botany as a career.

Section 1--Laboratory Techniques

Exercise I

Objective: To isolate pathogenic organisms, in pure culture, from diseased plant tissue.

Materials: Diseased plant tissue, sterile nutrient agar, scalpel, forceps, 70% alcohol, Petri dishes, alcohol lamp. thermometer, heavy cloth, 5 mm loop transfer needle, sterile distilled water and commercial "Clorox".

Procedure:[2] Method I. Tissue Transplants. Wash the diseased plant tissue to remove as much debris as possible. Cut small sections of diseased tissue from the advanced margin of the lesion, dip in 70% alcohol, and sterilize

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[2]A. J. Riker and Regina S. Riker, Introduction to Research on Plant Diseases, (St. Louis, 1936), pp. 44-46.

the tissue by submerging it in a 1:10 dilution of "Clorox" in distilled water. The length of time of this treatment depends upon the nature of the tissue. Heavily cutinized thick material will require a considerable longer treatment than thin delicate material. This time range should be within 1 to 10 minutes.

Using a sterile forceps, (sterilized by dipping in alcohol and flaming) transfer the tissue from the disinfectant to a sterile water wash. Transfer, aseptically, to a sterile agar plate. Four or five tissue sections may be applied to the same agar plate.

Method II. Dilution Plates. A suspension of bacterial or fungus cells obtained from the lesion is mixed thoroughly in varying dilutions with separate lots of melted agar, previously cooled to about 45°C and placed in sterile Petri dishes. To prepare the dilution plates, place 1 cc of suitable liquid nutrient solution in each of 4 Petri dishes and pile these one upon another on a wet cloth in front of the operator. To make the suspension for the first dilution, place one loopful of solution in the top dish with a flamed 5 mm loop transfer needle. Stir the loop of material in this solution and without flaming, move one loopful directly to the liquid in the second dish and stir. Likewise, without flaming, transfer a loop of solution from the second to the third dish and so on to the last dish. Pour in the desired amount of melted and cooled (45°C) agar and swirl to mix the agar with the solution in

the plate.

After either or both of the above techniques have been completed, store the Petri dishes at room temperature in a place where they will be as free from contamination as possible. Observe the growth which appears after a few days. Using a sterile needle, transfer, under aseptic conditions, hyphal segments to other sterile agar plates in an effort to isolate the organism in pure culture.

Exercise II

Objective: To experiment with several methods of inoculating plants with pathogenic organisms.

Materials: Various pathogens in pure culture, susceptible plants, corn-meal sand medium, polyethylene bags, sterile water, atomizer, hypodermic syringe and needle, rubber tubing, transfer needle and carborundum.

Procedure:[3] Artificial inoculation can be made in a variety of ways which more or less simulate natural inoculation. Several methods are described below, the procedure will depend upon the pathogens available.

Method I. Planting in "Inoculated" Soil. This method is effective when dealing with Fusarium wilt pathogens. Aseptically transfer the pathogens to sterile corn-meal sand medium (see appendix) and incubate at room temperature for 15 days. Mix the corn-meal sand culture with soil in the proportions of 1 to 5 and incubate at room temperature

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[3]Ibid., p. 55.

for 15 days. Select a susceptible plant and, when planting, place about 100 grams of the "inoculated" soil around each plant.

Method II. Dusting. This method is used in applying certain fungus spores to the host. The procedure depends upon the pathogen. For wheat seeds, dust with smut spores before planting. For fungi causing rust and mildew, dust the leaves of the susceptible host. Since adequate moisture is desired for leaf infection, refer to procedure 3 described under Method IV.

Method III. Spraying. Place a susceptible plant, to be inoculated, in a humidity chamber (polyethylene bag covering the plant and tied at the bottom) for 24 hours. From a pure culture plate or tube, make a suspension of fungus spores or bacteria by adding sterile water, swirling and pouring off the suspension. Remove the plant from the moisture chamber and using a hand atomizer, spray the plant thoroughly with the suspension of the pathogen. Return the plant to the moist chamber for 24 hours, remove and allow the plant to develop symptoms.

Method IV. Wounding. The three procedures described in this section introduce the pathogen into the host by means other than that which might be of a more natural nature. Suspensions of diseased tissue particles and small portions of the culture are used as well as spores and bacterial cells.

Procedure 1. Using a suspension of bacterial cells

or fungus spores in a hypodermic syringe equipped with a needle (size varied according to need), inject a healthy susceptible plant.

Procedure 2. Modify the hypodermic syringe by removing the needle and inserting a 1 inch piece of rubber tubing over the outlet. Fill the syringe with the pathogenic suspension and, applying the rubber tipped syringe to the underside of the leaf of the plant and holding the thumb tightly over the portion of the leaf covering the rubber tip, force the suspension into the intracellular spaces of the leaf. This treatment results in a water soaked appearance which disappears after a few hours.

Procedure 3. Place a pot of wheat plants in a humidity chamber for 24 hours. Remove the pot and rub the leaves with carborundum and then rub wheat leaf rust spores on the leaves. Return the pot of plants to the humidity chamber for 24 hours. At the end of this period, remove and set in a well lighted window at room temperature until symptoms appear.

Section 2--Demonstrating Pathogenicity

Exercise I

Objective: To demonstrate the pathogenicity of bacteria.

Materials: Plant material infested with bacterial blight of cotton (Xanthamonas malvacearium), cotton seed, Petri dishes, sterile water, "Clorox", alcohol, scalpel, forceps, polyethylene bags, hypodermic syringe, rubber tubing, alcohol lamp and sterile nutrient agar.

Procedure: Review all available literature concerning the pathogen being studied. Study diagrams and pictures and make drawings to become familiar with the morbid anatomy of the host plant as well as the organism itself.

Isolate the organism and grow it in pure culture as described in exercises 1 and 2 of Section 1.

Plant 3 pots of seeds of a variety of cotton known to be susceptible to bacterial blight of cotton. When the plants are well established and before the cotyledons become heavily cutinized place the plants in a humidity chamber, made from the polyethylene bags, for 24 hours before inoculation.

Prepare the inoculum as follows: To the pure culture of the pathogen, add ½ test tube of sterile water and swirl to make a suspension of bacterial cells. Inoculate one pot of plants by using each of the following methods.

Method I. Modified Hypodermic Syringe. Using the technique outlined in Section 1 introduce the inoculum into the intracellular spaces of the cotyledon.

Method II. Spraying. Follow the procedure as outlined in Section 1.

Expose the third pot of plants to the same conditions but do not inoculate these as they serve as a control.

Observe the plants periodically and when symptoms are well established, isolate from the infected area, grow the pathogen in pure culture and inoculate a second set of healthy susceptible plants by the same procedure as outlined

above. This is basically the procedure followed in carrying out Koch's Postulates and should be followed in any study of a pathogen which can be grown on artificial media.

Exercise II

Objective: To demonstrate the pathogenicity of a facultative fungus.

Materials: Plant material infested with the cotton wilt fungus (Fusarium vasinfectum), Petri dishes, sterile water, "Clorox", alcohol, scalpel, forceps, nutrient agar, test tubes, transfer needle, hypodermic syringe, corn-meal sand agar and seeds of a susceptible variety of cotton.

Procedure: Make a literature review as indicated in exercise I. Plant three pots of seeds of a susceptible variety of cotton about 2 weeks before conducting the expiment so as to insure well established growth.

From a diseased plant, isolate and grow in pure culture the pathogen by the method outlined in exercise I of this part. When the organism has been isolated in pure culture, use the following methods of inoculation.

Method 1. Soil Inoculation. As outlined in exercise 2, transfer the isolated pathogen to a corn-meal sand medium and incubate at room temperature for 15 days. Mix the corn-meal sand culture with the soil around the roots of one pot of the susceptible plants. Place in a well lighted window, water as needed and allow 2 to 4 weeks for symptoms to develop.

Method 2. Inoculation with Hypodermic Needle. Examine

the pure culture at intervals for spores. When sporulation has occurred, pour in about ½ test tube of sterile water and swirl the tube. Fill the hypodermic syringe, equipped with needle, with the spore suspension and inject into the stems of the test plants. Set the plants aside in a well lighted window, water as needed and allow 3 to 4 weeks for symptoms to appear.

Set a third pot of plants aside as a control giving it the same treatment as the others but do not inoculate the plants with the pathogen.

At the end of the 3 to 4 week incubation period, examine all plants for external symptoms. Make a diagonal cut through the stem and examine for the typically discolored vascular tissue. Compare the inoculated plants to the control.

Isolate from the infected plants, grow the pathogen in pure culture and inoculate a second set of healthy susceptible plants by the procedures outlined above.

Exercise III

Objective: To demonstrate the pathogenicity of an obligate fungus.

Materials: Fresh plant material infested with wheat leaf rust (Puccinia triticina), susceptible variety of wheat plants and polyethylene plastic bags.

Procedure: Make a literature review as indicated in exercise I. Plant 2 pots of a variety of wheat known to be susceptible to wheat leaf rust. When the plants are

about 2 weeks old or well established, place both pots in a humidity chamber for 24 hours. At the end of this time remove both pots of plants. Inoculate one pot of healthy plants by using spores taken from the diseased leaves. Remove the spores by passing an infected leaf between the thumb and index finger. Inoculate a leaf of the healthy plant in the same manner. Continue this procedure until several leaves of the healthy plants have been inoculated. A small amount of carborundrum previously rubbed on the leaf will help insure inoculation. Return the pot of inoculated plants to the humidity chamber. Treat the control plant in the same manner but do not inoculate. After 24 hours, remove both pots from the humidity chamber and place them in a well lighted window. Continue to water and observe each day for symptoms.

Exercise IV

Objective: To demonstrate the pathogenicity of a virus.

Materials: Source of wheat mosaic virus (Marmor tritici), mortar and pestle, susceptible plant and carborundum.

Procedure:[4] Make a literature review as indicated in exercise I. Plant 2 pots of wheat of a susceptible variety and grow for about 2 weeks or until the plants are well established. Prepare an inoculation extract by triturating fresh diseased tissue with a sterile mortar and pestle.

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[4]Ibid., p. 60.

Rub this extract lightly over the leaf of the plant employing just enough friction to produce a slight mechanical injury. Fingers, cheesecloth, cotton swab or similar material may be used as the spreader. Better infection may often be secured with virus difficult to transmit if fine abrasive material (e.g., carborundum powder 370 grain) is dusted on the leaf before it is rubbed. Allow about 10 days to 2 weeks for symptoms to develop. When symptoms appear, compare the diseased plant to the other pot of plants which is the control.

PART V

GENETICS

Introduction

Genetics, the science of heredity, is among the newest of the biological sciences and has developed rapidly during the last sixty years. Due to its application to humans, as well as domesticated plants and animals, this field of science is of widespread importance to anyone who desires a true insight into human nature and the living world at large.

The study of genetics has been left primarily to college and university classes with little, if any, attention given it at the high school level. There is, however, a need for the teaching of genetics in the high schools. Many students never enter college, therefore they never have an opportunity to explore this increasingly important field. Most high school biology courses introduce the science of genetics to the student but place little emphasis upon it although there may be students who are genuinely interested in this subject. For these students who have the interest and initiative to make a further study of genetics, this part has been included.

The exercises listed here are designed to better acquaint the student with genetics by using methods which are readily translated into understandable terms. The problem method is emphasized leaving terminology and technique to develop as the problems progress.

The simplicity of these exercises should not mislead the student into thinking the study of genetics is an easy one. On the contrary, a thorough understanding of many sciences is necessary for advanced work in genetics.

Although genetics is a relatively new field of science it is one which is being rapidly explored, consequently, more and more questions arise with every piece of evidence which is discovered. The answers to these questions and the uncovering of still more lies in the hands of the investigators of the future--the students of today.

Exercise I

Objective: To demonstrate albinism in maize.

Materials: About 100 grains of maize carrying the factor for albinism (see appendix), ordinary flat or wooden box and sand to fill the box.

Procedure:[1] Plant about 100 grains of the maize in moist sand in the flat or wooden box. Within 8 to 12 days seedlings will develop sufficiently to show green and albino plants. The two kinds may be counted and the numbers

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[1]David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences. (New York and London, 1938), pp. 407-409.

compared. The ratio of green to albino is approximately 3:1. Usually this exact ratio is not obtained, but is approached. There are greater chances of obtaining the exact ratio with a large number of seedlings than with a small number.

If space is available and time permits, the seedlings may be grown to maturity and the seed may then be used to produce the F_2 generation. To do this, plant grains from an experimental lot of albino maize in good garden soil and cultivate as for ordinary corn. The albino plants die within 2 or 3 weeks. When tassels and ears on the green plants begin to appear, cover and tie a paper sack over the tassel and ears before stamens and silks appear. When the pollen is shedding in the sacks over tassels, vigorously shake each sack-covered tassel to release as much pollen as possible. Remove a sack with pollen and dust it over the silks of an ear or ears of the same plant which have been covered. Replace sacks over the ears to prevent entrance of foreign pollen, leaving them for about 10 days or longer. Be sure to tag each self-pollinated plant so that it may be recognized at harvest time. After the corn is dry, and before using experimentally, a few grains from each ear must be given a germinating test. This is to eliminate the pure line green which is of no value for demonstration purposes. Save only those ears of which some grains produce albino seedlings.

Exercise II

Objective: To study dominant and recessive hypocotyl color in jimson weed.

Materials: 100 hybrid (F_1) jimson weed seeds, flat or shallow wooden box and soil or sand to fill the box. See appendix for seed source.

Procedure:[2] Plant the seeds in moist soil and care for as in the case of any other seedlings. Seedlings will develop sufficiently to show characters and for counting within 8 to 12 days. The hypocotyl (region from soil line up to the cotyledons) will show a purple color in about 75 per cent of the plants and green in about 25 per cent (3:1 ratio).

Exercise III

Objective: To demonstrate dominance, segregation and the selection of a pure line in coat color of mice.

Materials: Two or more pairs of tested mice with the formulas of CCBB and ccBB (such tested mice may be obtained from General Biological Supply House, Chicago, Ill.), cages to keep the pairs separate and feed.

Procedure:[3] To Show Dominance: Mate the mice in such a manner that a white mouse and a black mouse always constitute the members of a pair. This cross is CCBB (black) X ccBB (albino). All the offspring from such a cross must of necessity be CcBB, which is hybrid black.

To Show Segregation (the 3:1 ratio). By inbreeding the

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[2]Ibid., p. 409.

[3]Ibid., pp. 409-410.

males and females of this F_1 generation the F_2 generation will produce both black and albino mice. If the number of offspring in the F_2 generation is sufficiently large the ratio will be 3 black to 1 albino.

To Show How a Pure Line is Selected. By mating any pair of the albinos from the F_2 generation a pure line of albino mice is established at once. To select a pure black line would take lnger but can be done by repeated matings of a back-cross type.

Exercise IV

Objective: To demonstrate dominance, segregation and a method of selecting a pure line in Drosophila melanogaster.

Materials: Cultures of wild type D. melanogaster and a culture of vestigial-wing-type, several small jars about the size of ½ pint milk bottles, banana, cotton to plug jars, knife, oven or double boiler and baker's yeast. The cultures of D. melanogaster may be obtained from a biological supply house.

Procedure:[4] Preparation of media: Make some balls of cotton large enough to fit snugly into the mouths of the culture jars. Plug the jars and heat at 160°C in the oven for one hour or longer. Sterilize the knife at the same time. Remove and cool slowly. Do not remove the plugs from the jars until ready to add the food. Select a ripe banana that has no breaks in the skin. Peel it carefully so as not to allow the fruit to touch anything. Cut about 2 inches in small

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[4] Ibid., pp. 410-412.

pieces from the peeled part and drop them into a culture jar. These pieces should be crushed into a pulp in the bottom of the jar using the sterile knife or a flamed glass rod. Drop a few drops of yeast culture on the banana and replace the plug. The plug must not be laid on a dirty table while the jar is being prepared or it will carry mold into the culture.

To Show Dominance. Remove about 10 flies from a jar of wild-type D. melanogaster. Anesthetize these and examine them with a hand lens or microscope and select several male flies. They may be distinguished from females by the clump or patch of dark bristles on the first legs. Place these males in a tiny cone twisted from clean paper and drop the come of flies into the culture jar. To etherize flies pour a few drops of ether on a piece of cotton and press it over the mouth of the culture bottle. As soon as the flies drop to the bottom and do not move, remove the cotton and dump the flies on a piece of paper or in a dish for examination. If the ether is applied too long the wings extend above the fly's back and this is an almost sure sign of death. Repeat this procedure using a culture of vestigial-wing D. melanogaster, selecting several females instead of males. Be certain to use a new culture so as to get the flies within 8 to 10 hours after they emerge from the pupae. The females must be virgin or the experiment is useless. Keep this new stock jar in a place where it will not be disturbed for a week. By this time the pairs will have bred and there

may be numerous tiny maggots in the bananas. The best temperature is about 23°C. In another week the maggots will have pupated and emerged, therefore it is important that all the pairs of parent flies be removed from this new culture jar by the time that pupae are first found on the sides of the jar. This new emergence represents the F_1 generation which is all hybrid for the long- and the vestigial-wing character. Since the normal, or long wing, is dominant, all the F_1 generation have long wings. This is contrasted with the yellowish-gray body of the wild. The wild type is again dominant so all the F_1 flies have yellowish-gray bodies. This gives two chances for dominance and recessiveness to be illustrated in a single cross.

To Show Segregation. From the hybrid culture just mentioned above withdraw about 10 adult flies. This must be done while the culture is quite young, that is, during the first week, or before they have had time to produce a second generation. Transfer the 10 flies to a new culture jar and when larvae or pupae appear on the jar remove the parent flies allowing the new generation to reach the adult stage. When adults have appeared in rather large numbers remove most of them from the jar and anesthetize or kill them. Be sure to shake those from the jar which seem more sluggish than the rest since they are vestigial winged and cannot move about so readily as the long-winged ones. Count the number of flies having long wings and compare the number having vestigial wings. The ratio will be close

to 3 long to 1 vestigial.

To Show How a Pure Line is Selected. Select males and females with the vestigial wings from the above culture and make a new cross in a fresh jar. If allowed to run for several generations, the offspring are all vestigial winged. This is a simple way of selecting a pure line from a mixed culture and illustrates the principle of selective breeding in the production of varieties of domestic stock. To show the difficulty involved, mate pairs of the long-winged flies from the segregated culture above. Some of these matings will produce pure-line long-winged cultures but most of them remain hybrid, producing vestigials in F_1 or F_2 generations.

PART VI

SUMMARY AND CONCLUSIONS

The need for personnel trained in botany and botanical research is one which can be fulfilled only by interesting the student of today in this increasingly important field of science. Before this is possible, however, they must first be motivated, encouraged and introduced to the opportunities therein.

The exercises proposed in this report include a variety of different subjects within four of the major fields of botany. The student should be allowed to choose a subject in which he is interested. He should then review all the available literature on the subject to become familiar with the problem, work it out thoroughly and draw logical conclusions. It is the opinion of the author that this method of teaching will introduce scientific research to the student and encourage him to continue the study of botany.

Although the scope of this report is limited, its purpose is to serve as an introduction to the study of botany and botanical research for the high school student.

Properly used, it is believed that this type of exercise can be of value as a means of creating interest in plant science.

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APPENDIXES

APPENDIX A

NUTRIENTS AND MISCELLANEOUS APPARATUS

Table I: Nutrients.

Shives Nutrient Solution: 49.01 gm KH_2PO_4 in 1 liter, 24.56 gm $Ca(NO_3)_2 \cdot 4H_2O$ in 1 liter and 73.95 gm $MgSO_4 \cdot 7H_2O$ in 1 liter. Fifty milliliters of each of these stock solutions plus 4 or 5 mg ferric phosphate and water to make 1 liter forms a full nutrient solution.

Nutrient Agar: 1000 cc distilled water, 17 gm agar, 3 gm beef extract and 10 gm peptone. Melt the agar in the water by heating in a steamer or an autoclave. Add the other ingredients and restore to volume with water if necessary. When all is in solution, adjust reaction, filter, tube, plug, and autoclave.

Corn-meal Sand Medium: 1500 cc distilled water, 1000 cc corn-meal and 1000 cc washed white sand. The amount of water varies with the quality of the corn-meal and the amount of water in the sand.

Table II: Miscellaneous Apparatus.

Red Light: To obtain red light cover the material with red cellophane and place it about 12 inches from a fluorescent light ("standard cool" bulbs are most suitable).

Far-red Light: To obtain far-red light, cover the material with one sheet of red and one sheet of blue cellophane and expose to light from a 300 watt incandescent bulb held in a reflector. Do not place material closer than 2 feet to this light source because of excessive heat.

Table III: Source of Living Materials.

Maize Seed: Meyers Hybrid Seed Corn Company, Hillsboro, Ohio, or from Dr. George S. Carter, Genetics Laboratory Supplies, Clinton, Conn.

Jimson Weed Seed: Dr. George S. Carter, Genetics Laboratory Supplies, Clinton, Conn.

APPENDIX B

REFERENCES TO CAREER OPPORTUNITIES IN PLANT SCIENCE[1]

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*NRSSP--National Roster of Scientific and Specialized Personnel.

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[1]Russell B. Stevens, Career Opportunities in Biology, (Evanston, Illinois and White Plains, New York), p. 61.

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