## Chapter 8

### **Teaching Botany Through Inquiry**

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#### INTRODUCTION

Introductory Botany is taught in two different formats at the University of Oklahoma. The first format includes a traditional lecture for 250 students each semester with inquiry-oriented laboratories of 36. The second format, from which most of the following material is taken, is taught completely using inquiry with 48 students in each class. There are three or four inquiry sections each semester, and each has a faculty member or an advanced graduate student in charge. The inquiry sections meet four times a week for 50 minutes each day. In these, lecture, laboratory and discussion are combined, with emphasis on laboratory and discussion. Students are given all the laboratory equipment and materials needed for each See pages labeled Day 8 - Day 13 from the lab prep day's lesson. manual which outlines what students and instructors need each day. Pages 27-36 are taken from the student's workbook which coincides with the activities that take place in class. Students are given reading assignments, but these and the workbook activities arc completed AFTER the students have participated in class. This insures that the "discovery" aspect of the inquiry method is Introductory Botany is not a content course but a maintained. concept course where students are trained to ask the right questions and to develop botanical/biological concepts on their own. Students are asked to learn facts, however, not as many are given as in a traditional lecture. Inquiry botany is designed for both majors and non-majors, although a majority of students are non-majors.

#### FIRST-DAY INQUIRY ACTIVITIES FOCUSING ON THE SCIENTIFIC METHOD

First-day activities focus on the "scientific method" which serves as the foundation for all inquiry activities throughout the semester. Although not all instructors or scientists agree on the existence of the scientific method or on its elements, I emphasize the following steps:

- 1) observation of a phenomenon
- 2) asking questions about the cause of the phenomenon
- 3) hypothesis formation
- 4) prediction about what will happen if contributing factors to the phenomenon are changed
- 5) experimentation to test the hypothesis
- 6) collection and analysis of the data
- 7) rejection or support of the hypothesis

Using The Scientific Method.

To the students: Suppose you are an objective observer watching an empty theater before the movie begins. You observe a young woman enter the theater by herself and sit in one of the seats. A few minutes later, a young man enters the theater and, without saying anything to the woman, sits down beside her. These are your OBSERVATIONS. Now, based on these observations, what QUESTIONS might you ask about this situation? Students give a variety of responses including: "Do they know each other?" "Is that the best chair for viewing the movie?" "Is he trying to pick her up?"

We can choose any one of these questions and study it by first forming a hypothesis and then testing the hypothesis. To FORM A

HYPOTHESIS, we can convert a question (for example, the first one asked) into a statement – The two people in the movie theater know each other. It this statement is true, then we can manipulate the situation and PREDICT what will happen. For instance, we could move one of the two people to a different seat (you can't ask the people questions) and see if the other person follows. If these two people do know each other as we have stated in our hypothesis, then the other person should follow. We can now conduct the EXPERIMENT and move one of the people to a different seat. If we move the woman, and the man follows, we need to write this down as our DATA. If we continue to move the woman from seat to seat and the man follows each time, then our HYPOTHESIS IS SUPPORTED. But, that does not prove our hypothesis to be correct – we cannot prove a hypothesis to be true, we can only support or reject it.

Students who are asked why the results of the experiment do not prove our hypothesis to be true provide responses such as: "He could be a masher following her", "He could be interested in her perfume and not her," "He might be trying to find the best chair in the theater," etc. This leads to further hypothesis formation and testing.

Review the scientific method with the students and point out the steps that were used in the discussion about the movie theater.

Followup First-day Activity.

This second activity also revolves around the scientific method. Duplicate and cut up the sheet of figures (Grouping Geometric Figures) and place one set of figures, except Q and R, into an envelope. Students are divided into teams of 2,3 or 4

students. Each team gets one envelope with 17 figures in it (A-P and S). The students should introduce themselves and then be given the following task: "Arrange the 17 figures into groups. You can have as many groups as your team wants, but you need to write down a statement describing why you have placed the figures in each particular group."

On the board, teams write the letters in each of their groups, but not the descriptions of the groups. The instructor chooses two interesting groupings (for example, the groupings of Team 1 and Team 4) and divides the class in half. One half of the class arranges their figures like those of Team 1 while the other half arranges their figures like Team 4. (Team 1 arranges the figures like Team 4 and Team 4 arranges like Team 1.) These are OBSERVATIONS. Students now try to decide why Team 1 (or 4, whichever is appropriate) arranged the figures as they did, and they write down the reasons. This is the HYPOTHESIS. The instructor now hands out figures *Q* and R and the students TEST THE HYPOTHESIS by placing Q and R into the groups they think Team 1 (or 4) would.

In a general discussion, students state why they think Team 1 (or 4) arranged the figures into their groups and into which groups they think Team 1 would place Q and R. Team 1 now reveals whether the discussion has been accurate. If so, the HYPOTHESIS IS SUPPORTED. If not, the hypothesis is not supported and must be rejected. Frequently, Q and R will be placed in the right groups, but for reasons other than those Team 1 (or 4) originally stated. In this case, the hypothesis is supported, but the hypothesis is

incorrect. This is an important point for discussion. Summarize emphasizing the steps used in the scientific method.

#### INQUIRY LABORATORIES ON LEAF ANATOMY AND PHOTOSYNTHESIS

Each student/workshop participant is given a prepared microscope slide of a privet leaf cross section and asked to make observations about the arrangement of cells. Students make a sketch of the leaf, and examples are drawn on the board. The instructor points out that it appears the lower group of cells within the leaf (spongy parenchyma) appears to be loosely packed and asks how we might be able to determine if this is true. Several responses are given including: "Observe leaf sections cut at different angles to see it cells are widely spaced in these views as well," "Stain cells so they more easily seen." If no one comes up with a desired response, the instructor asks a "prompting question"-- "How do we know that air is in a balloon?" A response -- "By squeezing or pushing the air out." A similar demonstration is done by placing a fresh privet leaf in very hot water. When this is done, bubbles form at the bottom of the leaf but not at the top. The instructor asks, "What information does this provide?" Several responses "The cells in the bottom halt of the leaf are loosely include: packed and surrounded by a gas that escapes from the leaf." Secondly, "There are holes in the bottom of the leaf through which gases can escape (or enter)." Finally, "There are holes in the bottom of the leaf but not the top." The instructor asks the students to look at the privet leaf cross section again and see if these holes (stomata) can be found. A discussion is held about the holes and the gases that might enter or leave.

Through a series of experiments and demonstrations, the students now develop for themselves the formula for photosynthesis. The instructor first shows the clearing and staining process that reveals the presence of starch within a leaf (hot water, hot alcohol, iodine). A discussion is held after each of the following experiments/demonstrations is conducted.

Experiment 1: Plant 1 kept in the light for 48 hours; Plant 2 kept in the dark for the same length of time. A leaf taken from Plant 1 stains darkly, the leaf from Plant 2 does not stain. Conclusion: Light is necessary for starch formation.

Experiment 2: Leaf 1 is placed in a bowl of glucose solution for 48 hours; Leaf 2 is placed in a bowl of water for the same length of time. Both leaves are kept in the dark. Leaf 1 stains darkly, Leaf 2 does not stain. Conclusion: Light is not necessary for the formation of starch, but glucose is. Secondly, light is necessary for the formation of glucose.

Experiment 3: We know glucose has carbon in it, but from what source does the carbon come? Plant 1 is kept under a sealed glass bell jar with a container of potassium hydroxide which removes all of the carbon dioxide from the jar; Plant 2 is kept under a sealed glass bell jar but with no potassium hydroxide. A leaf from Plant 2 stains darkly, but a leaf from Plant 1 does not stain. Conclusion: Carbon dioxide is necessary for the formation of starch (and glucose), and the carbon in the glucose comesfrom carbon dioxide. Experiment 4: How does the carbon dioxide enter the leaf? Plant 1 has half of the top of each leaf coated with vaseline and Plant 2 has half of the bottom of each leaf coated with vaseline. Leaves

from Plant 1 stain darkly throughout the leaf while leaves from Plant 2 stain darkly only in the half of the leaf where there was no vaseline. Conclusion: carbon dioxide (or some other substance necessary for photosynthesis) enters the leaf from the bottom of the leaf. Instructor should relate this information back to the study of leaf anatomy and previous experiments.

Demonstration 5: Is chlorophyll necessary for photosynthesis? Both albino and normal green corn seedlings are tested for starch. Only the green corn seedlings are shown to produce starch. Conclusion: chlorophyll is necessary for photosynthesis. Discuss importance and role of chlorophyll.

Demonstration 6: Separation of spinach leaf pigments using paper chromatography. Demonstrates that chlorophyll is not the only pigment in leaves. Discuss role of all pigments in a plant. Demonstration 7: From what source does the hydrogen in glucose come? Discussion is held about an experiment involving heavy water and a green plant in sunlight. This demonstrates that water is necessary for the production of glucose, and that the hydrogen in glucose comes from water.

Experiment 8: What else, beside glucose, is produced in photosynthesis? Elodea Plant #1 is placed in water under light; Elodea Plant #2 is placed in water in the dark. Funnels arc inverted over both of the containers and a test tube is placed at the end of each funnel. Test tubes inverted over the containers holding the elodea are tested with a glowing splint. The splint glows brighter when placed in the test tube over Elodea #1 and does

not glow brighter when placed in the tube over Elodea #2. Conclusion: oxygen gas is produced in photosynthesis.

A discussion is held to re-emphasize major points and to recreate the formula for photosynthesis. Four to five class periods are used for the development of the formula for and the discussion of photosynthesis.

#### KEYS TO INQUIRY

1. Lead students to discover the biological and botanical concepts for themselves rather than just telling them. For instance:

<u>Traditional Approach</u> A fruit is a ripened ovary containing seeds.

#### Inquiry Approach

What do the following structures have in common? squash, tomato, apple, watermelon, peanut, etc.

Make a list of responses and then discuss.

Note: Inquiry takes longer than the traditional approach.2. Incorporate elements of the scientific method into your course as often as possible. Most importantly, allow students to make

observations, to form hypotheses, and to test hypotheses through experiments or demonstrations.

Note: Active discussions are essential to the success of this method of instruction.

3. Use a variety of approaches for each concept you teach--experiments, demonstrations, living materials, discussion of data already collected, films, and slides. You don't have to do an experiment to do inquiry.

4. It is possible to adapt traditional laboratories or demonstrations so that students can discover concepts on their own. Rule of thumb--Are you telling students information that they could figure out for themselves? Can you give students just enough information about a concept so they can work out the rest?
5. Start asking questions on the first day and encourage discussion from the very beginning.

6. Think up questions you will ask and responses students nay give BEFORE you get into the classroom. By doing this, you can control the direction of the discussion. This requires that you think through the day's activities. You will find that discussions often stray from your intended path, but as long the discussion is productive, that's okay. Your prepared questions will allow you to get back on track whenever you need to.

7. Ask only one question at a time. Do not ask "yes or no" type questions or any question that has only one correct answer--such as "Does this leaf stain positively for starch?" You <u>should</u> ask open-ended questions to which there are several responses.

 Don't answer your own questions. Wait for answers from students -- even if it seems like an hour before you hear a response.
 If no students respond, rephrase the question.

9. Accept all responses made by students – don't be negative as long as the students are making a legitimate attempt at contributing to the discussion.

Try to get several answers to each question you ask.

10. Summarize every day. With inquiry, students sometimes complain that they don't know what notes to take in class. These students often seem to be the ones who are passive, who don't participate, and who just want answers.

11. Try to get everyone involved!

12. If you meet every day, do not use inquiry ALL OF THE TIME. Students will become annoyed at being constantly asked questions--mix your teaching methods.

# **Grouping Geometric Figures**

В D G Ε Н L 0 Ν M P S Q R

Cut apart the figures on this page. Then sort the figures into groups.

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#### TISSUE CONCEPT AND EXTERNAL TISSUE OF LEAVES

#### Each Student:

1. Microscope

#### Each Two Students:

- 1. Dissecting tray
- Dissecting tray
   One potted <u>Graptopetalum</u> plant with large number of leaves (for epidermis)
   Finger bowl with 12 wilted leaves of wandering jew (wilted for two days) picked from bed in moderately bright light
- 4. Reagent rack with dropper bottle of water

#### Demonstration Table:

1. Same as for students

#### Greenhouse Order:

- 1. 25 Graptopetalum plants
- 2. Many (300) wilted wandering jew leaves

#### Grocery Order.

1. Nothing

#### LEAF STRUCTURE USING PRIVET

#### Each Student:

- 1. Microscope
- 2. Slide set series "A" (slide #4-A, X sec. privet leaf and #3-A, epidermis of wandering jew for review purposes if necessary)

#### Each Two Students:

- 1. Dissecting tray
- 2. Dropper of water in wooden rack
- 3. Fresh microtomed X sec. of privet leaf, in small Syracuse watch glass of water. They should be cut about 60-90 microns thick. The basal half of the leaf will give much better midveins. (Keep in cold room and make sure they are thin enough. Check with microscope.) Put 10-15 young privet leaves between 2 potato or carrot slices, then microtome.

Use potato for microtoming. Use sliding microtome in Room 19.

4. Bottle of water containing two 10' leaf bearing stems of privet. (Wide mouthed 1 pint bottle) (for macroscopic observation of the leaf)

#### Demonstration Table:

- 1. Same as for students
- To demonstrate the release of gas from the inter-cellular spaces of the leaf:
   a. Potted coleus and geranium plant with leaves containing air in the intercellular space. (2 plants each)
  - b. Electric hot plate
  - c. Glass 400 ml beaker of hot water. (Start to heat before the 9:30 a.m. section)
  - d. Pair of long (10" or 12") forceps
  - e. Asbestos gloves
- 3. Rotary microtome (in Room 156)
- 4. Leaf skeletons to demonstrate veins
- 5. Leaf model

#### Assistants:

1. Check the supply of privet sections each hour and be sure for thickness during the time they are microtomed. Do not cut too thick.

#### Greenhouse Order:

- 1. Fifty 10" leaf hearing privet stem tips
- 2. Twoeach coleus and geranium

#### Grocery Order:

**1.** 1 potato

#### Prep Notes:

- 1. Set up coleus leaf blades in sucrose and in water 48 hours ahead of testing. (for DAY 11).
- 2. Set up vaseline coleus 48 hours ahead of testing (for DAY 12).
- 3. Prepare large glob of gluten without starch (for DAY 10). Soak large glob of gluten in beaker of water for three or four days. <u>Periodically knead and rinse gluten under running water during this time</u>. (Gluten is prepared by adding water to powered gluten to form a dough-like consistency.)

<u>Day 10</u>

#### CHEMISTRY AND THE CHEMISTRY OF CARBOHYDRATES

#### Each Student:

- 1. Microscope
- 2. Slide set series "A"

#### Each Two Students.

- 1. Dissecting tray
- 2. Reagent block with
  - a. Dropper bottle of water
  - b. Dropper bottle of iodine (Sufficient concentration to give a positive test for starch grains)
- 3. Carbohydrates in small bottles: potato starch, glucose, fructose, and sucrose. (Samples will be taken from the bottles of sugar and starch, therefore, it will be necessary to check and refill many of the bottles before they are stored after their use) (to test for solubility).
- 4. Microtome sections of a potato tuber in a Syracuse watch glass of water. The sections should be between 100 and 200 microns thick. These sections should be kept in a refrigerator over-night. Be sure to check the sections occasionally as they are being cut to ascertain their fitness for study. Use sliding microtome in Rm. 19 - add a few drops of lemon juice to preserve slices.
- 5. Corn, olive, coconut, cottonseed oils
- 6. Gluten in finger bowl of water

#### Demonstration Table:

- 1. To demonstrate a test for starch in potato tubers:
  - a. One potato tuber per section
  - b. One sharp knife for slicing potatoes
  - c. One 125 cc. dropper reagent bottle of iodine solution
  - d. Two clean petri plates per section
- 2. Corn or oat seedlings 6" to 8" tall that have been growing in:
  - a. the dark in white quartz sand
  - b. the light in white quartz sand (to show that, initially, seedling gets energy from seed.)
- 3. Large glob of gluten without starch (test a small glob for starch-should be starch-negative)
- 4. Beaker with water

#### Greenhouse Order:

1. Corn or oat seedlings growing in quartz sand and seeds of each.

#### Grocery Order:

1. Large potatoes - 2 per section

#### Prep Notes:

- 1. Set up carbon dioxide coleus one day ahead of testing (for DAY 12)
- 2. Prepare gas free water (for DAY 12) by boiling H<sub>2</sub>0 and let it sit there for days.

#### FOOD MANUFACTURE IN PLANTS --PHOTOSYNTHESIS

#### Each Student:

1

Nothing

Demonstration Table:

- To demonstrate the effect of light on starch formation:
  - a. Varigated coleus plant that has been growing in light, displayed on the demonstration table <u>under a</u> light with leaves which show a good starch test.
  - b. Varigated coleus plant that has been growing in continuous darkness until the leaves are completely starch-free (5 to 10 days in a dark box)
    c. Plant with leaf mask
- 2. To demonstrate the synthesis of starch in the dark by destarched coleus leaves floating on a sugar solution
  - a. Support blade of leaf on rim of petri dish with petiole extending into a 4% sucrose solution. Place under dark box for 48 hours before needed for class. These leaves should be starch positive on the veins only.
  - b. Control for above except that water is used in place of a sugar solution. The leaves should be <u>starch negative</u>
- 3. Equipment for testing for the presence of starch in leaves. (For procedural demonstration only). The following list of materials will be referred to here after as "Leaf Starch Testing Equipment":
  - a. Hot plate and extra hot plate with asbestos pads
  - b. 2 beakers each: 600,400, 250, 250 ml
  - c. One 1 liter bottle of 95% ethyl alcohol
  - d. One 500 cc bottle of iodine solution
  - e One 500 cc. bottle for waste alcohol
  - f. One pair of long forceps (10" stainless steel)
  - One box of starch free filter paper, to fit petri plates
  - g. One box of star h. Six petri dishes
  - i. Enamel tray of water and one enamel tray empty
  - j Three or four standard lantern slide cover glasses on which to float leaves.
  - k Box of soda crackers (Matzos). Large square of cheese-cloth
  - 1. 1 asbestos glove
- 4. Labeled dentonstrations of leaves from coleus plants listed above (tested for starch):
  - a. Untreated
  - b. Boiled in water
  - c. Boiled in water and alcohol
  - d. Boiled in water and alcohol and treated with iodine solution.

The above demonstrations should be displayed on moist filted paper in a covered petri dish. A complete set "a-d" should be made for one plant above, but only a "d" demo should be prepared for all other tests.

5. Instructors may run tests on the plants on the light and dark plants. Be sure plants have enough leaves for all sections, or see that replacement plants are available.

#### DAY 11 Continued on Next Page

#### <u>Assistant</u>s

1. Return all demonstrations to the instructor's desk and be sure to replenish the supply of 95% ethyl alcohol on hot plate.

#### Greenhouse Order:

- 1. Seven 9" high varigated coleus plants (in 3" pots) from bright light. These should be strongly starch positive.
- 2. As above except from the dark box and should be starch negative.
- 3. One plant with leaf mask (see Dr. Uno).

#### Grocery Order:

1. See grocery order, Day 25

#### FOOD MANUFACTURE IN PLANTS: Photosynthesis Cont'd

#### Student's Table:

1. Nothing

#### Demonstration Table:

- 1. To demonstrate the liberation of oxygen by Elodea in bright light:
  - a. Place six 6" stem tips of Elodea beneath a glass funnel with the cut end directed toward the funnel stem. Place the funnel and <u>Elodea</u> in a 1 gallon battery jar filled with gas free water. Support the rim of the funnel from the bottom of the battery jar with three #9 rubber stoppers. The level of the water should cover the tip of the funnel. Place this apparatus in bright light such as a group of flourescent lights. Oxygen should be liberated from the stem tips and will collect in the test tube. Fill elodea collection tube with  $0_2$  from tank. Replenish  $0_2$  for each section.
  - b. Control: Same set-up as above except that it will be displayed under a small dark box. No oxygen will collect in this test tube if prepared properly. Check test tube each hour.
- 2. To demonstrate that carbon dioxide enters the leaf mainly through the stomates:
  - a. Starch-free variegated coleus plants with one half the underside of each leaf coated with vaseline. Place plant in bright light. (A series of fluorescent lights should be used as they will not melt the vaseline.) They should remain under this light for at least 48 hours or until the uncoated side becomes strongly starch positive (black). Remove vaseline with toluene.
- 3. To demonstrate that carbon dioxide is necessary for photosynthesis:
  - a. Expose three starch-free variegated coleus plants in the following environments (label such that students can't see the labels).
    - 1. Variegated coleus plant from the dark box exposed to bright light until the leaves give a positive test for starch.
    - 2. Variegated coleus plant from the dark box sealed under a bell jar from which all of the carbon dioxide has been removed by a strong solution of calcium hydroxide. This solution may be placed in a beaker containing a fluff of gauze which will increase the reacting surfaces. Caution: Do not allow the calcium hydroxide to overflow onto the table. The above plants should be starch negative.
    - 3. Same as above except that instead of removing the carbon dioxide with calcium hydroxide a 5% carbon dioxide gas should be added to the bell jar. Include a small beaker of water with a gauze wick as #2 above for a control. This plant will become strongly starch positive
  - b. A kip carbon dioxide generator with attached wash bottle (Don't seal the tube or top of the CO<sub>2</sub> generator)
  - c. The bell jars used in the above experiments should have openings in the top to permit removal of the leaves with long forceps.
- 4. Starch testing equipment from previous lesson
- 5. Labeled demonstrations of coleus plants listed above, tested for the presence of starch. Only one demo per plant is necessary
- 6. Box of salt-free crackers (Matzos, from preceding day)
- 7. Wooden splints and matches

#### DAY 12 Continued on Next Page

#### Greenhouse Order:

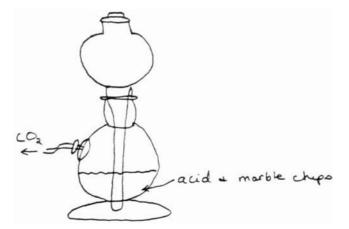
- 1. More coleus plants as needed from the light and dark
- 2. 12 6" elodea stem tips

#### Grocery Order:

1. Nothing

#### Prep Notes:

- 1. Soak 30 wheat seeds overnight for pyrogallic demo. (for DAY 16)
- 2. To renew  $CO_2$  generator: Open the valve until all acid has drained into the bottom and middle sections. Carefully lift off the top section and empty out acid. Replace the acid with a 50% HCl and 50% water solution. (NOTE: add acid to water and work under a hood). Fill to one third of middle section. Don't fill up to stopper on side. Carefully spoon in about half a cup of marble chips (CaCO<sub>3</sub>) into the middle chamber. Replace the top section and make sure it is well sealed. After a few seconds, close the valve to stop the reaction.



#### FOOD MANUFACTURE: Photosynthesis and Chlorophyll

Each Two Students.

- 1. Potted, variegated Coleus plant
- 2. To demonstrate separation-of pigments by paper chromatography
  - a. Dropper bottle of spinach chlorophyll ground in acetone and filtered
  - b. 500 ml bottle containing 1 inch of solvent Pet Ether fitted with cork containing hook which holds a 2 inch wide strip of chromatography paper long enough to touch surface of solvent.
  - c. I capillary tube

Demonstration Table:

- 1. To demonstrate that photosynthesis occurs in chlorenchyma:
  - a. One variegated coleus plant, under a light, with starch in the leaves. This plant should have a large non-green central area.
  - b. One purple Coleus plant with starch in the leaves, under a light. Leave the lights on in the room overnight.
- 2. Labeled demonstrations of leaves from Coleus plants listed above tested for a) untreated, b) boiled in water, c) boiled in water and alcohol, d) boiled in water, alcohol, and treated with iodine solution. This demonstration should be displayed on moist filter paper in a petri dish.
- 3. Leaf starch-testing equipment. (See DAY 11)
- 4. A labeled demonstration of the chlorophyll separation in a large cylinder of sufficient size to be seen at the back of the room.
- 5. One small flat of albino corn seedlings from the light (in green house).
- 6. One or two plants with variegated leaves with some leaf areas non-green.

#### Assistants:

1 Return all demonstration material to its proper place on the demonstration table. Check the supply of all the reagents and if low be sure to replenish the supply.

#### Greenhouse Order:

- *1.* 28 8"-12" variegated Coleus plants
- 2. 2 purple Coleus plants
- 3. 3-4 different plants with variegated leaves, some areas non-green.
- 4. 1 flat of albino corn

#### Grocery Order:

- 1 1 pound frozen spinach.
- 2. 1 pint karo (obtain from stockroom)
- 3. 2 packages yeast (obtained from the micro-stock room)

#### Prep Notes:

- 1. Start pyrogallic acid demo (see DAY 16)
- 2. Set up week old yeast culture (for DAY 17). Add 1 pint karo and 2 packages of yeast to 3000 ml of water.
- 3. Start 4 cups of wheat seeds germinating about three or four days before needed (for DAY 16). Germination is somewhat faster under a light.

#### DAY 13 Continued on Next Page

- 4. The chlorophyll separation in a cylinder is kept in the refrigerator. When this needs renewed apply the following instructions:
  - a. Fresh chlorophyll extract. Can be prepared by boiling green leaves (frozen spinach) in 80-85% alcohol.
  - b. Cylinder of 50% chlorophyll extract and 50% petroleum ether. (demonstrated separation of chlorophylls from carotinoids). Add petroleum ether to chlorophyll extract and shake. Remove stopper carefully to release pressure buildup. Add a little water and continue shaking. When shaking stops two layers will form. The upper layer should **be** green and the lower one yellow.

#### LEAF CELLS AND LEAF TISSUES

1. Stripoff the skin, or epidermis, of a sedum leaf. What is the color of the epidermis? How many cell layers thick is the epidermis? Based on its location, what might be the function of the epidermis?

Why do you think the tissue beneath the epidermis is called chlorenchyma?

2. With the microscope, examine pieces of the upper and lower epidermis of a zebrina leaf. How many different kinds of cells do you see in the epidermis?

Label the kinds of cells in the top diagram on the next page.

#### STOMATA

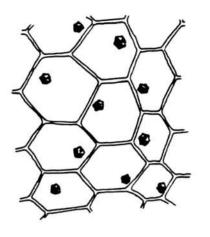
The stomate (plural, stomata) is a small opening between each pair of guard cells. Are the walls of the guard cells of uniform thickness on all sides of the cell? \_\_\_\_\_\_\_ How might this help the guard cells open or close the stomate?

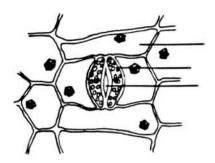
What are the small green bodies in the guard cells?

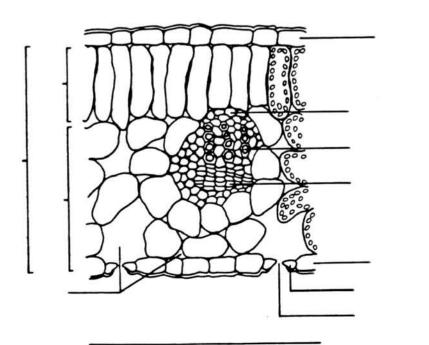
Are stomata present in both the lower and upper epidermis of the leaves of the zebrina plant? \_\_\_\_\_\_ Approximately how many stomata are present in each square millimeter of epidermis from the lower leaf surface? \_\_\_\_\_\_ How many stomata in the upper epidermis? \_\_\_\_\_\_\_ (The visible portion of the leaf under the low power of the microscope is approximately one square millimeter in area.) The number of stomata per square millimeter of leaf surface varies in different parts of a leaf surface and also in leaves from different plants as shown by the following table.

Average number of stomata per square millimeter of leaf surface

Plant	In upper epidermis	In lower epidermis
Elodea	0	0
Coleus	0	141
Nasturtium	0	130
Black Walnut	0	460
Kidney Bean	40	176
Sunflower	85	156
Oats	25	23
Corn	70	88
Tomato	12	130
Water Lily	460	0







- 3. What do you think is the significance of the different number of stomata in the top and bottom leaf epidermis?
- 4. In what kind of environment do you think you would find plants with very few stomata on their leaves?
- 5. Examine the cross section of a living leaf with the unaided eye and with a microscope. Compare your observations with a specially prepared slide of a similar leaf. Do all the cells in the leaf look the same? \_\_\_\_\_ Describe the differences you see.

How many groups of related cells can you identify? \_\_\_\_\_ Each group of similar cells is called a <u>tissue</u>. These may be similar in position (as in epidermis) and similar in function (as in phloem). Locate the following tissues in your leaf section and then give its function.

Epidermis Chlorenchyma Palisade parenchyma Spongy parenchyma Phloem Xylem	Name of tissue	Function
Palisade parenchyma Spongy parenchyma Phloem	Epidermis	
Spongy parenchyma Phloem	Chlorenchy ma	
Phloem	Palisade parenchyma	
	Spongy parenchyma	
Xylem	Phloem	
	Xylem	

Identify the epidermis, palisade parenchyma layer, the spongy parenchyma layer, the phloem and xylem of the bottom diagram on the previous page.

#### PLANT FOOD

Three types of sugar are used by plants as food. They are glucose (grape sugar);
fructose (fruit sugar); and sucrose (cane sugar). Starch is also used by plants, but is
made up of many linked sugar molecules. Place a small amount of starch in your hand
and taste it. In the same way taste glucose, sucrose, and fructose, in this order. Then
taste glucose again. List the three sugars and starch in the order of their sweetness.
1) 2) 3) 4) Place a very small amount of glucose, fructose, sucrose, and starch on different
corners of a slide, keeping track of where you placed each. Examine them all under
the low power of the microscope. Add a drop of water to each and observe again.
What happened to the glucose?
What happened to the fructose?
What happened to the sucrose?
Were they destroyed? Are they still sugar? How could you separate
them again from the water? What happened to the starch?
Why do you think so?
Add a drop of iodine solution to each of the sugars and starch on your slide, observe,
and record the results for 1) glucose
2) fructose 3) sucrose

2. Food classification. Name some of the foods you eat, and based on their main chemical content, place them in one of the following categories. Carbohydrates:

Fats:

Proteins:

Which of the above categories of food can provide energy for you? What are some of the uses you make of the energy and molecules that are chemically bound in food?

How does the plant get this food?

4) starch

3. Food observation within plant cells. Place an extremely thin fresh section of potato in a drop of water and observe using the low-power objective of the microscope. Add a drop of iodine solution from the edge of the cover glass and observe again. Record your observations.

Does the potato contain starch? \_\_\_\_\_ How do you know?

How can you account for empty cells?

#### FOOD SYNTHESIS IN PLANTS: PHOTOSYNTHESIS

- a. Why do molds grow luxuriantly on bread in a dark box, but green plants die in good soil in a dark box?
- b. Why do the non-green parts of green plants die if the green parts are removed as rapidly as they begin to grow?
- c. Why do albino seedlings in good soil die about three weeks after germination, while green seedlings continue to live?
- d. Why do underground organs of plants in good soil die if the tops above ground are kept from developing?

These and many more questions will be answered by this section about photosynthesis.

#### TEST FOR STARCH

Using a leaf from a coleus plant kept in the sunlight, the instructor will:

- 1. place the leaf in boiling water to remove the purple pigment (anthocyanin).
- 2. place the leaf in hot alcohol to remove the chlorophyll.
- 3. treat the leaf with iodine to determine if starch is present.

The instructor will repeat the previous three steps on a leaf from a coleus plant which has been in a dark room for several days.

Record your observations about the leaves taken from the two different conditions, and the effect of light on the presence of starch.

LIGHT

DARK

What conclusion can you draw about the effect of light on the presence of starch?

A leaf from a coleus plant kept in the dark has been immersed in a 4% solution of glucose. Similarly a leaf from the same plant has been placed in water without sugar. During the treatment, both leaves were kept in the dark for 24 to 48 hours. Using the test for starch above, check for the presence of starch, record your observations about the amount of starch present in the leaves under the two conditions.

4% SUGAR WATER \_\_\_\_\_

WATER \_\_\_\_\_

What is your conclusion about sugar and the formation of starch?

Is light necessary for the change of sugar to starch in leaves?
Is light necessary for the synthesis of sugar in leaves?
Which of these two processes do you think is photosynthesis?
How can you determine if your data and conclusions about coleus leaves are true for
leaves in general?
Under what conditions might starch become changed to sugar in a plant cell?

To demonstrate the liberation of gas during photosynthesis, a short-necked funnel has been placed over some <u>Elodea</u> in a jar and a test tube inverted over the funnel. The whole apparatus has been placed in the light. The gas that replaces the water in the test tube will be tested with a glowing splint. Draw a diagram of the experimental apparatus and explain what is happening, i.e., for what are you testing with the glowing splint and how was it produced?

To determine if any of the components of air are necessary for photosynthesis, we will use two plants that first have been placed in a dark room for several days to insure that no starch remains in the leaves. One of these plants was placed under a bell jar along with a bottle of KOH which removes carbon dioxide from the air. The bell jar was then sealed to a glass plate with vaseline. The other plant was left uncovered and both plants were placed in the light. After two days the leaves of both plants are tested for starch using the previously described starch test. Record your observations for this experiment.

COVERED

UNCOVERED

Are there any of the components of air necessary for photosynthesis? Explain your answer.

What gas do you think is required for photosynthesis? Into what plant molecule is this gas incorporated?

Does gas enter the leaf, mainly through the stomata or directly through the epidermal cells? To answer this question, a coleus plant is used because nearly all its stomata are in the lower epidermis of the leaves. The lower surface of some of the leaves are covered with vaseline and the plant placed in the light for several days. Now we will remove the vaseline from the leaf and test for starch. Record your observations.

LEAVES WITH VASELINE

#### LEAVES WITHOUT VASELINE

Does gas enter the leaf through the leaf epidermis or through the stomata?

How can you tell?

Write the chemical equation for photosynthesis, indicating both the materials used and the products produced.

#### PAPER CHROMATOGRAPHY

Basic research in modern cell biology helps to determine the metabolic processes that occur, their significance, and how each activity may affect other activities. Learning more about metabolism frequently requires the isolation and purification of chemicals extracted from cells. Paper chromatography is one technique useful in separating the components of cell extracts. In paper chromatography, chemicals are carried on filter paper by a solvent. Because different chemicals have different solubilities in the solvent, the chemicals will separate from each other as they travel on the filter paper. To demonstrate chromatography of a leaf extract:

- 1. Filter paper is trimmed so a strip will fit into a test tube without bending.
- 2. Application of spinach leaf extract is made about 3 centimeters from the end of the strip and allowed to dry.
- 3. Step Two is repeated several times, with application of the extract to the same place each time, until a large amount of extract has been applied to a small spot on the paper.
- 4. Test tubes are filled with a small amount of solvent and supported in an upright position.
- 5. The strip of paper is inserted into the prepared test tube with the treated end down so it dips into the solvent but the spot remains above the solvent. The test tube is stoppered, making sure not to move the test tube during the experiment.
- 6. Make observations and a diagram showing the results, showing which pigments are present and which ones travel fastest.

The relationship of photosynthesis to light intensity is stated in the table below. The first column lists the percentages of light intensity compared to that on a clear July day. The other vertical columns in the table represent the relative rates of photosynthesis in the designated plants when exposed to the differing light intensities. Plot the data on the graph below, drawing a curve for each of the five plants.

Relative rates of photosynthesis expressed in milligrams of

Light Intensity:

Spinach         Potato         Nasturtium         Pine           2         5.5         0.3         0.0         00           5         7.1         2.5         1.1         0.1           10         8.0         5.0         2.1         0.6           20         8.9         6.4         3.7         1.2           30         9.6         7.0         4.6         1.6           40         10.0         7.3         5.1         1.8           50         10.4         7.6         5.4         2.1           60         10.7         7.7         5.6         2.3           70         10.8         7.8         5.8         2.7           90         11.0         7.8         5.8         2.7           90         11.0         7.8         5.8         2.9			square meters of leaf			percentages of full sunlight
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Fern	Pine	Nasturtium	Potato	Spinach	
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	0.0					2
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	0.8	0.1	1.1	2.5	7.1	5
$\left \begin{array}{c c c c c c c c c c c c c c c c c c c$	1.6					
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$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	1.9	2.5	5.7	7.8	10.8	
	1.8	2.7	5.8	7.8	11.0	
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Light intensity: Percentage of Full Sunlight

In general, how do changes in light intensity affect the rate of photosynthesis?

Which plant	would	grow	best	in	full	sunlight?		_ In	dim	light?
			pine	or	the fe	rn do better	in full sunlight?			In
dim light? _										

The rate of photosynthesis in a potato in relation to temperature and the concentration of carbon dioxide in the air is represented in the table below. Draw a curve showing the rate of photosynthesis for each concentration of carbon dioxide.

Temperature in degrees	5	10	15	20	25	30	35	40	45
Rate of photosynthesis at 0.03 percent $CO_2$	2.1	6.5	8.6	13.8	8.8	8.0	5.7	2.9	0.0
Rate of photosynthesis at 1.22 percent $CO_2$	2.9	12.4	20.2	28.1	33.7	56.0	48.3	35.6	8.0

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In general, how do changes in temperature affect photosynthesis?

How does an increase in  $CO_2$  concentration affect photosynthesis?

#### SUMMARY

- 1. What kind of "food" is used by plants and how is it used?
- 2. Explain, in your own words, the process of photosynthesis.
- 3. What factors in the environment affect photosynthesis?
- 4. How do humans make use of the knowledge of the relationships between food manufacture in the plant, growth and development of a plant, and environmental conditions?

5. Scientists grew plants using water molecules that contained a radioactive isotope of oxygen, <sup>18</sup>0. Here is the resulting formula:

 $C^{16}0_2 + 2H_2^{18}0 - (CH_2^{16}0) + {}^{18}0_2$ 

Can you determine which is the molecule from the left side of the equation that yields the oxygen gas on the right side? Explain your reasoning.

- 6. What are the main products of the light-dependent reactions (light reactions) of photosynthesis?
- 7. What are the main products of the light-independent reactions (dark reactions) of photosynthesis?