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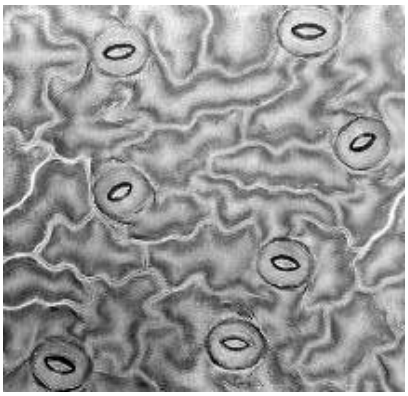
EXPERIMENTS

Environmental Correlates of Leaf Stomata Density

Bruce W. Grant and Itzick Vatnick
Biology, Widener University, Chester PA, 19013
grant@pop1.science.widener.edu
vatnick@pop1.science.widener.edu



stomata viewed at 400x in nail polish impression from leaf underside © Marc Brodkin, 2000

ABSTRACT:

In this three week lab, students use the technique of making clear nail polish impressions of leaf stomata to generate and test an hypothesis of their choice about how leaf stomata density might vary under different environmental conditions. First, students learn how stomata density affects leaf carbon, water, heat budgets, and photosynthesis. Then, students design their own study to compare stomata density among leaves that differ in biophysical environment on their campuses. Over the next two weeks, students collect and analyze their data (graphs and t-tests), and present their results in an in-class symposium.

Table of Contents:

KEYWORD DESCRIPTORS:

Principal Ecological Question Addressed: Effects of biophysical environmental conditions on the adaptive design of plant leaves.

Ecological Topic Keywords: plant physiological ecology, biophysical ecology, environmental adaptation, stomata, photosynthesis, autecology.

Science Methodological Skills Developed: field work, hypothesis generation and testing, microscopy, statistics, graphics, data analysis, scientific writing, oral research presentations.

Pedagogical Methods Used: student-directed inquiry, cooperative learning, problem-based learning, scoring rubrics.

CLASS TIME: MULTIWEEK - three 3 hour lab periods

OUTSIDE OF CLASS TIME: 4-12 hours during which students collaborate to generate their written reports and prepare for their oral presentations.

STUDENT PRODUCTS: Students are assessed based on their oral and written presentations, their written responses to background questions about plant stomata and other topics, on their written stomata research proposal, on their data collection and management skills, and on their participation in discussions in the stomata results symposium.

SETTING: Outdoors on campus for data collection (any season - even mid-winter), back in lab for data analysis.

COURSE CONTEXT: Undergraduate freshmen biology and other science majors (course name: Introduction to Organismal Biology - Bio 162), 12-16 per lab section.

INSTITUTION: Metropolitan private primarily undergraduate university.

TRANSFERABILITY: It could be modified to run in any undergraduate introductory biology lab course (major or non-major) at any college or university. It also could run in introductory lab courses in botany, ecology, environmental science, or upper division courses in a variety of sub-disciplines. It could also be modified to run in biology lab courses in grades 8-12.

SYNOPSIS OF THE LAB ACTIVITY (audience: students)

WHAT HAPPENS:

In this three week lab, students use the technique of making clear nail polish impressions of leaf stomata to generate and test an hypothesis of their choice about how leaf stomata density might vary under different environmental conditions. First, students learn how stomata density affects leaf carbon, water, heat budgets, and photosynthesis. Then, students design their own study to compare stomata density among leaves that differ in biophysical environment on their campuses. Over the next two weeks, students collect and analyze their data (graphs and t-tests), and present their results in an in-class symposium.

LAB OBJECTIVES:

At the conclusion of this multiweek lab, students will:

1. students will have a basic understanding of structure and function of leaf stomata as well as the role of stomata in regulating gas and heat exchange in vascular plants,
2. students will have actually done science - they will have generated a testable hypotheses, collected data, analyzed data, tested their hypothesis, and they will have reported their research results to their peers.

EQUIPMENT/ LOGISTICS REQUIRED:

* live plant material (of your choice),
* clear nail polish and clear plastic package tape,
* clean slides, marking pen, scissors, plastic slide holder,
* microscope and stage micrometer,
* computers with spreadsheet, presentation, and basic statistical software.

SUMMARY OF WHAT IS DUE:

From this multiweek lab, students submit

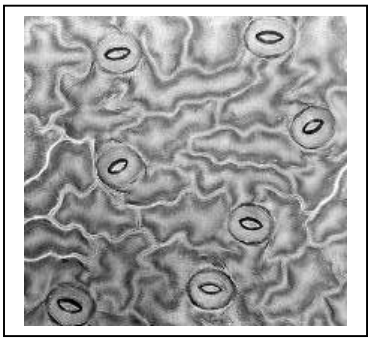
- a one page co-authored research proposal composed according to the guidelines below (due at the end of the first lab),
- answers to any four of the questions for further thought contained in this handout (due in lab on the second week - students work alone on these),
- clearly labeled copies of students' original data including the actual slides taped to a plastic slide holder (due in class at the end of the second lab),
- co-authored stomata results report composed according to the guidelines below and presented in class (due at the beginning of the third lab), and
- a critical review of the lab activity (due one week after the third lab - students work alone).

DESCRIPTION OF THE LAB ACTIVITY

INTRODUCTION (written for students):

Leaf stomata are the principal means of gas exchange in vascular plants. Stomata are small pores, typically on the undersides of leaves, that are opened or closed under the control of a pair of banana-shaped cells called guard cells (see figure above). When open, stomata allow CO_2 to enter the leaf for synthesis of glucose, and also allow for water, H_2O, and free oxygen, O_2, to escape. In addition to opening and closing the stomata (stomata behavior), plants may exert control over their gas exchange rates by varying stomata density in new leaves when they are produced (such as in the spring or summer). The more stomata per unit area (stomata density) the more CO_2 can be taken up, and the more water can be released. Thus, higher stomata density can greatly amplify the potential for behavioral control over water loss rate and CO_2 uptake.

red maple leaf



stomata viewed at 400x in nail polish impression from leaf underside © Marc Brodkin, 2000

But why, you might ask, might it be adaptive for a plant to control its rates of water loss and CO_2 uptake? One answer can be found in the sun. Generally, plant photosynthetic apparati are only designed to function well over a rather narrow range of temperatures. When heated, cytochromes, pigments, and membranes critical to phosphorylation and carbon fixation rapidly denature (i.e., they cook). To avoid this, an individual plant may open its stomata and evaporate water which will lower the leaf temperature. Thus, one may hypothesize that leaves in the sun should have higher stomata density than do leaves in the shade - all else being equal.

But, on the other hand, if water is not available, such as under drought conditions, excessive evaporation might lead to desiccation and an equally severe disruption of photosynthetic function. Thus, one might expect plant leaves exposed to drought conditions to have fewer stomata in sunlit environments.

The above discussion illustrates a very important concept in experimental biology - there are often alternative hypotheses to explain variation in nature. In this case, stomata density may increase or decrease in response to environmental variation in sunlight and water availability. Note that since you will not be measuring sunlight or water availability you should use caution in how you word your acceptance or rejection of your hypothesis for your plants.

MATERIALS AND METHODS.

Study Site(s).

Plant samples for this lab are to be collected from plants on campus within a few minutes walking distance of class. Despite that Widener University is in urban Chester, PA, and that we do this lab in mid-winter, it is not a problem for students to find plants with green leaves for their studies. This includes ornamental evergreen ground cover plants, shrubs, and trees (such as grasses and weedy perennial forbs, hollys, yews, and conifers). The activity does not work well on dried plant material, because it is a bit tricky, but not impossible, to obtain the stomata samples (see below).

Data Collection Methods.

Week 1.

Envision an environmental difference that might affect stomata density and formulate an hypothesis about which way you would expect stomata density to vary and WHY. Discuss these in detail with your lab instructor PRIOR to taking any data. Next, decide on a place anywhere within about 10 minutes walking time where you intend to collect leaf samples in the environmental types of interest, and go and get them. Bring your leaf samples back to lab and count their stomata densities (see Methods for Obtaining Stomata Impressions below). Lastly, submit your co-authored research proposal with your partner. This document should fit on one page and should contain three sections according to the Guidelines for Stomata Research Proposal below.

Week 2.

Next week, bring all of your data to class, finish counting stomata (if you have not already have done so), and your instructor will help you with the statistical analyses, and computer graphics generation of your stomata data to test your hypothesis (see Guidelines for Data Analysis below). In addition, you should begin to produce your oral and written reports which are due the following week.

Week 3.

The entire lab period this week will be devoted to a symposium of presentations of your research results to your peers. You and your research partner will make a 12 minute oral report to your peers using visual aids (such as an overhead projector and/or video projector for a PowerPoint presentation, see Guidelines for Oral Presentations below). Also on this date, your co-authored written report is due (see Guidelines for Written Reports below) as well as your disk copy of your data (see Guidelines for Data Management below). Your individually written critical review of this multiweek lab activity is due the following week (see Guidelines for Reflective Reviews of Lab Activities below).

Methods for Obtaining Stomata Impressions.

1. Obtain the leaf upon which you wish to census stomata.

2. On the side you wish to census stomata (typically the leaf underside) paint a rather thick swath of clear nail polish.

3. After the nail polish has dried (several minutes), obtain a square of VERY CLEAR tape (such as package sealing tape, but do NOT use scotch tape). Stick your tape piece to the area that contains the dried nail polish swath.

4. GENTLY, peel your nail polish swath from the leaf completely. You will see a cloudy impression of the leaf surface now attached to your tape piece (hereafter referred to as your "leaf impression").

5. Tape your leaf impression to a VERY CLEAN slide and use scissors to cut off the excess tape.

6. Use a pen and write some sort of ID code signifying the treatment group name (e.g. leaf from sun) and other info (e.g. leaf #3) directly on the slide.

7. Focus your leaf impression under at least 400x power and observe the stomata (see image above).

8. Search around on your impression to find an area that subjectively appears to have a high density of stomata. That is, move the slide around until the field of view is away from the edge of the impression and so that there are no dirt blobs, no thumbprints, no damaged areas, and no big leaf vein impressions in view.

9. Count all stomata you see and record the number neatly on a clearly labeled data sheet. (Note that you should design a data sheet on which to record your stomata counts that clearly indicates which data correspond to which leaf and treatment group. You will be separately assessed on how neatly you accomplish this part of the task.)

10. Repeat the previous two steps three times, and the highest number of the three will be your one datum from this impression.

11. Repeat all steps above for at least 12 different leaf impressions in each treatment group.

Your instructor will demonstrate the use of a stage micrometer so that you may convert your data from units of "stomata number per field of view at 400x to units of stomata per mm; Since there are subtle differences among microscopes in the exact size of the field of view you must convert your data to units of stomata/mm {Hint #1: recall that the area of a circle = pi * radius^2. Hint #2: your measurement of the area of the field of view at 400x should be about 0.12 mm^2. If your answer differs, ask for help}.

Detailed Guidelines.

Guidelines for Stomata Research Proposal.

At the end of class after the first lab period, you and your partner should submit your research proposal. This document should fit on one page and should contain three sections:

Introduction: two sentences in length and beginning with "We propose to investigate the effects of ....[describe the environmental difference] ... on leaf stomata density in ... [plant names etc.]. The second sentence should begin with "We hypothesize that stomata density in the.... should be greater because in this environment .....

Methods: one brief paragraph describing EXACTLY where you will go to locate plant individuals to sample (draw a little map), and EXACTLY from where on each individual plant the leaf samples will be collected. For this lab, we will require that you collect at least 12 different leaves (we will call these replicates) from each environmental type to calculate test statistics such as "average stomata density" in each of the environmental types of interest.

Possible Results: one brief paragraph and one clearly labeled figure of hypothetical data that would visually provide an answer to your hypothesis (i.e. what would a graph look like if your hypothesis were true or were false?). Your paragraph should begin with something like "If our hypothesis were correct..." or "If it is the case that that under such and such [fill in your choice] .... environmental conditions, then leaves with higher stomata density should be favored because [then, explain WHY...]... " Following this, state something like... "therefore we would expect our data to reveal a pattern such as in Figure 1: Hypothetical Data...." The next sentence should say, "However, if our hypothesis were not correct... if other factors that we don't understand were strongly affecting stomata density... [etc.], then we would expect our data to show ..... see Figure 1..." Obviously, make sure that one can tell which pattern is which in your figure.

There are lots of ways of crafting the prose in this section. The point is for you to offer a clear proposal of what your results should look like if the environmental difference of interest were to affect stomatal variation in the manner you hypothesize.

Guidelines for Data Analysis.

After you have collected your stomata data you are ready to test your hypothesis. Enter your data in a spreadsheet available in lab (such as Microsoft's Excel). Find the averages, standard deviations for your data groups. Also, construct a graph summarizing your stomata results.

Consult with your instructor if you have questions about graphics generation using available software and about exactly what statistical test is best for your data; however, in our experience, data from the vast majority of projects may be analyzed using a t-test.

We have created a detailed PDF of "Appendix 1: Guidelines for Statistical Analysis" of your stomata data that includes information on basic descriptive statistics and the t-test. Please read these pages carefully and consult with your instructor if things are still unclear.

Guidelines for Written Reports.

You have not done science until you have presented your data and interpretations in a way that is usable by your colleagues. Dozens of books have been written on how to write a research paper, how to write a thesis, etc. Although it is true that the style and content of most scientific papers are fairly consistent, it is not true that good scientific writing is dry and dull. Good writing is catalytic to learning and understanding, and your development as scientists (whether or not you choose a career in science) requires proficiency in oral and written communication.

At the beginning of Week 3 you and your research partner will submit your written report and you will present your research results to your peers in an in-class symposium. Your written report should conform to a standard format for scientific papers that contains the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Literature Cited (if any), and an Appendix containing the original data. Each section serves a specific and unique function, the details of which are given in the PDF "Appendix 2: Detailed Guidelines for Stomata Lab Written Reports"

Guidelines for Oral Presentations.

1. You should always compose the written report first, and then distill salient features for your 12 minute oral presentation (with a 3 minute Q/A session).

2. Oral reports should contain 4 sections each of which serves a specific function:

    * Introduction (3 mins), (Note: CLEARLY STATE YOUR HYPOTHESIS)
    * Materials and Methods (3 mins),
    * Results and Specific Discussion of Them (3 mins), and
    * General Discussion of Results and Future Research Directions (3 mins).

3. The principal differences between oral reports and written reports are that:

    * oral reports do NOT start with the Abstract, they start with the Introduction,
    * oral reports do NOT detail the methods as extensively,
    * oral reports present the results and offer brief discussions interpreting the results as they are presented, whereas written reports only discuss results in the Discussion section.

4. You should think very carefully about how to use visuals (overheads, computer projectors, etc.) to convey your findings, and you are encouraged to use presentation development software (such as Microsoft's PowerPoint [however, select colors that work well together with NO animations or sound effects]).

5. NEVER read your talk, however, neither should you ad lib. Use a normal speaking voice, address your audience (NOT to the blackboard or projector image), and explain what you asked, what you did, what you found, what it means, and what you would do next to follow up. Rehearse your talks at least three times!

6. Lastly, you will lead the 3 minute Q/A session after your talk, during which your peers will be asking you questions. Since good questions and their answers are rewarded, your task is to move things along and answer clearly and succinctly. Encourage the more silent students in the class to engage in the discussion.

Guidelines for Data Management.

Your research team will be given a data disk on which you will keep all of your data, analyses, Tables and Figures, and the current version of your manuscript (you must provide a backup disk). *** WARNING *** beware of swapping disks while running MS-Word or Excel - you might get a lockup and lose all unsaved information. We suggest saving all work on the hard disk (or ram disk) of whatever computer you are using (such as in the “My Documents” folder), and then every 10 minutes while working and when done, use MS Windows Explorer to copy your work to your principal and backup disks on the A: drive. Ask if you are unsure about how to do this. You will turn in your data disks to your instructor when you turn in your report. There should be 3 files on your disk (1) your manuscript in MS-Word, (2) your Tables and Figures in MS-PowerPoint, a nd (3) your data in MS-Excel. Details follow:

(1) MS-Word manuscript file (*.doc extension):

* choose a file name prefix that consists of some abbreviation of your last names as one word of at least 8 characters in length,
* put your Abstract on the very first page using 12 point, single spaced text, and with your names and the title of your project at the top left of this page,
* begin each major section with a new page,
* remove all Tables and Figures from your Word file, however,
* list all of the Table and Figure legends in 1-2 pages after your Literature Cited section,
* your Appendix goes last,

(2) MS-PowerPoint presentation file (*.ppt extension):

* use the same prefix for your PowerPoint file name as for your manuscript,
* if you used PowerPoint for your Oral Presentation, then simply use your talk for this part of the assignment,
* if you did not use PowerPoint for your Oral Presentation, then this file should contain clean full page versions of each of the Tables and Figures you used in your manuscript - one per PowerPoint “slide” and in their numerical order as they appear in your manuscript.

(3) MS-Excel date file (*.xls extension):

* use the same prefix for your data file name as for your manuscript,
* clearly label each EXCEL worksheet with a useful name (e.g. “original data”),
* do not combine totally different data and analyses on the same worksheet,
* at the top of each worksheet type into a little text box a written explanation of what is contained in that worksheet, and, most importantly, the date of last update,
* imbed each Table or Figure in the worksheet next to the data it depicts,
* insert a text box containing the “Legend” from the manuscript for every Table, Figure, or statistical analysis you use in the manuscript,
* delete all outdated or inaccurate worksheets, analyses, Tables, and Figures; however, you are welcome to retain versions of figures that you did not use in the manuscript (provided that they are well documented, too),
* beware of making duplicates of data and then forgetting which version is the most current.

Guidelines for Reflective Reviews of Lab Activities.

No lab activity is perfect and its participants, YOU, are the best judges of what changes need to be made to make things run more smoothly so that you can get the most out of it. In addition, numerous studies have shown that students learn more if they are involved in the teaching process. This activity is specifically designed to meet both objectives: improve the labs and help you get more out of your time and effort here.

After the completion of this lab activity, we want you to write a brief reflective review. The text should be formatted double spaced, 12 point, 1" margins, and minimum 300 words in length. Your charge is to convey your most pressing concerns regarding the strengths AND weaknesses of the lab activity. In addition, explain exactly how the lab activity should be modified to improve it. Be constructive. For example, if the lab was in your opinion "too long" which particular activities would you omit and why? If an activity in a lab was "a waste of time," why was it so? Re-examine the objectives of the lab on the first page of the lab write-up. What specific lab objective(s) were or were not met, and what specific activity should be used instead that would accomplish these lab objective(s) that were not met? To repeat, your comments must be constructive to be given credit.

Your critical/constructive review of the lab activity is due at the beginning of the following Wednesday noon hour meeting. Clear, concise, and insightful reviews that demonstrate your reflection and constructive criticism of a lab activity will earn +20 points.

QUESTIONS FOR FURTHER THOUGHT AND DISCUSSION.

1) How exactly do stomata open and close? How do guard cells work? Specifically explain the roles of ions and any plant hormones.

2) As you will see in this lab activity, plants confronted with different environmental conditions vary the number of stomata per unit area by quite a lot. Yet, in theory the same result due to having more stomata could be attained by simply having bigger stomata with no difference in stomata number - however, plants vary stomata number and not stomata size. Why? Given your answer to Question (1), why might plants vary stomata density rather than stomata size?

3) Why might it be adaptive for stomata to occur mostly (if not entirely) on the undersides of leaves? What plants show the reverse pattern for which stomata are only on the upper leaf surface?

4) Some cacti thrive in some of the hottest deserts on earth where water is extremely scarce for most of the year. To deal with the scarcity of water, cacti have evolved an unusual set of adaptations including a remarkable capacity to soak up water into fleshy stems when it rains and hold onto this water during drought. One way cacti have to hold onto water is to ONLY open their stomata at night when it is cooler and more humid. However, if CO_2 is only allowed into these plants at night how are cacti able to synthesize sugar with it via photosynthesis during the day many hours later?

5) Diagram and describe some of the physical aspects of leaf design that would reduce water loss in a dry environment. Specifically address how leaf size, shape, orientation to the sun, color, fuzziness, thickness, water-proofing, stomata design, stomata density, etc., might vary from a wet to a dry environment.

6) Climate change due to the rapidly increasing levels of greenhouse gases (particularly CO_2) in our atmosphere is a serious current global concern. How might stomata density serve as a bioindicator for monitoring the response of plants to changes in greenhouse gas concentrations in the future? (Hint: what do the data say for how stomata density varies with CO_2 concentration?)

7) As a related question to the one above, how might stomata density serve as a bioindicator for estimating CO_2 concentrations in the past (paleoclimates)? Find and summarize two instances of research on this topic in the literature. (Hint: see references below, [F. Wagner's in particular].)

8) Given your knowledge of the tradeoffs plant leaves face between carbon dioxide uptake and evaporative water loss, speculate upon the "behavioral" features in stomata you would expect to evolve in plants adapted to dry environments with variable and unpredictable water supply. Research your answer and provide

support (with references) for any mechanism(s) that has(have) been identified as a way for stomata to "behave" in response to humidity and water availability.

9) Among bryophytes, stomata are restricted to the sporophyte life stage (found in mosses and some hornworts). Why? Why might it be adaptive for only the sporophyte and not the gametophyte stage in the life cycle to possess stomata?

10) Plant tissues are extremely sensitive to damage by the powerfully oxidizing effects of ozone (O_3. What effects would you expect this to have on urban-rural gradients in stomata density, and how would this effect interact with other urban-rural gradient effects on plants? What are the implications of these issues to urban agriculture?

11) Many bacteria and fungi that are parasitic of plants face the daunting task of finding and infecting a new host by airborne spore dispersal followed by germinating upon and then penetrating the leaf surface of their host. What are some of the specific adaptations possessed by some of these parasites to gain access to leaf tissue by entering through stomata thereby evading the plant leaf cuticle? (Hint: search on rust fungi, *Uromyces*.)

12) What role do stomata play in the solution to the problem of getting water up to the leaves from the roots of woody plants (which for a tall tree such as a redwood can be over 350 feet up!)? Using a little system diagram, sketch and describe the role of stomata in water uptake.

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Comments by Contributing Authors – Bruce Grant and Itzick Vatnick

CHALLENGES TO ANTICIPATE AND SOLVE.

We have identified 5 challenges that commonly arise:

1. correlation and causation: Many students do not understand the fundamental difference between correlation and causation. In this lab, stomata density variation likely results from interacting environmental factors (e.g. CO_2, temperature, water, etc.); therefore, higher stomata density might be consistent with a student's hypothesis about causation, but students need to understand that this does not allow one to say anything like "the data prove our hypothesis." We suggest that instructors offer a little talk on inductive/ deductive reasoning and hypothesis testing in science.

2. falling behind in the first 15 minutes: Many students seem to stumble into class without much of a clue about what is happening in lab on the day the stomata lab begins. The instructor must engage these people immediately - which is why we begin the lab with a "call and response" activity (see below).

3. little sense of direction: Many students do not really understand what an environmental difference is from a plant's perspective - also, they have very little conception of which way is "NORTH" and the degree to which a particular shrub on campus is "sunny" or "shaded" over the extended daylight hours other than the instant of their observation. In other words, few students really understand the path of the sun in the sky across their campus. Instruction about this should be done outdoors - not in class. Bring a globe.

4. statistics literacy: Most students have not seen the basic concepts of statistics we expect them to use in this experiment. The instructor must attend to these needs. For some non-majors or pre-college settings, it might be better to have students assess their differences in stomatal averages visually, perhaps using a more simplified short-cut, such as the two standard deviation rule.

5. presentation effects: Most students have never made a scientific presentation before. The instructor must attend to these needs, and in particular encourage supportive behavior by the peer learning community.

COMMENTS ON THE LAB DESCRIPTION.

Introducing the Lab to Your Students.

We introduce this lab using a "call and response" activity in which we ask the students to list and explain factors that affect the photosynthetic rate of a leaf. Two categories quickly emerge - characteristics of the environment (sunlight, air temperature, humidity, water and CO_2 availability) and characteristics of the leaf (CO_2 uptake capacity through stomata, water balance, and leaf size, shape, color, orientation, evaporative water loss rate as these affect leaf temperature).

We take an important moment to review a basic misconception found in many students. We point to a wooden table in the classroom and ask the students "where does most of the mass of this wooden table come from - the ground or the air?" Many say the ground, which then opens a discussion of what wood is (cellulose and lignin), what photosynthesis does (basic chemical equation to make sugar), and we remind them that the carbon that forms a huge component of the mass of wood, in fact comes from the air as CO_2.

Next, we specifically guide the discussion to an explanation of the means of plant uptake of CO_2 through leaf stomata - what stomata are, briefly how they work, and we review their roles in gas exchange with their environment (CO_2 and O_2 exchange, and H_20 loss). Stop and poll your students to recall from where the water comes that exits via the stomata (i.e., uptake at the roots). Next, we talk about principles of leaf design, and we project images of photos of different types of leaves (desert shrubs, tropical understory plants, etc.) to reinforce a few basic morphological themes in biophysical adaptation. Students are now thinking about leaf design and are ready to think about hypotheses that they can test with the plants on our campus.

Comments On the Activities in the Lab.

Try to move the students toward a novel project, but advise against taking on too ambitious a variation since the rate limiting step is always the drudgery of making the slides and counting stomata. The key consideration is to not let anyone out the door to collect their plants who does not have a very clear idea of what they are doing. We take the students outside as the next thing after the lab introduction, and we (1) show them which way is north, (2) we walk to and examine plants on the north and south side of the biology building, (3) we walk to and examine a few leaves from the upper south and lower north sides of a thick leafy shrub nearby (to demonstrate "sun" and "shade" leaves), and we show them that in fact you can easily see the morphologial differences among plants in different environments on campus. Next, we leave the students outside with the explicit understanding that they will return to class in 15-30 minutes for a second consult session to finalize their hypothesis, sampling sites, and study species BEFORE collecting plants. DO NOT SUGGEST A PROJECT FOR THEM TO DO.

Remind everybody which way is north - it never ceases to amaze us how disconnected many students are from basic compass directions; however, in our students' defense, we are all in an urban environment where there is little need to know this kind of thing (and in fact, a major interstate highway, I-95, officially runs "north" and "south" despite that it runs due east and west as it comprises the southern border of our campus).

Another consideration is about the plants on campus. Some students may not know that it is less than a good idea to cut daffodil leaves from beneath the window of the President's

office for their sample. Again, this reinforces the importance of the consult session with the instructor PRIOR to collecting plant samples.

Advise the students to collect a few extra leaves for trials to see if the nail polish works - in our experience, some of the brands of nail polish chemically react with some of the epicuticular waxes of leaves (especially so for holly leaves). If so, the nail polish will not harden. We suggest having several brands of clear nail polish on hand, one of which will undoubtedly harden to make the impressions.

Open the classroom windows, because nail polishes often contain some nasty smelling volatiles that should be ventilated. Circulate your Departmental policy on exposure to potentially toxic chemicals (this is especially important for students who have allergic sensitivities or who may be pregnant). If you do not have a written policy on this - get one!

Instructors should review basic microscope techniques. Assume very little retention about the details of handling, focussing, etc., with the scopes from previous courses.

Advise the students to select the magnification of choice based upon how many stomata they see. Flip to a higher or lower power so as to see about 20-60 stomata per field of view. More than 60 is too many to count, and fewer than 20 introduces too much variation.

Remind the students to apply the nail polish to the leaf undersides where stomata are generally the most dense. Ask you students why this might be so? However, if any of your students happen to select a species of grass, advise them to first find out which side has the most stomata, since some grasses show the reverse pattern (for which during development the grass blade flips over and grows upside down). This may also prove problematic with conifers and some monocots for which no clear "leaf underside" is apparent. The rule is to check first. Also, remind the students to avoid leaf veins when selecting areas to count stomata.

Instructors will have to demonstrate the use of a stage micrometer to estimate the size in mm2 of the field of view at various magnifications. Alternatively, the conversions could be worked out beforehand and averages determined for the microscope brands in your lab.

A great deal of very interesting and important statistical content has been omitted from the handout in Appendix 1. For example, there is no discussion of assumptions of normality, non-parametric tests, type 1 vs. type 2 errors, degrees of freedom, or anything about how the t-test critical values are calculated. Put this and other content back for some or all of your students at your discretion.

During the Stomata Symposium it is absolutely critical that the instructors abstain from dominance; in fact, we play only observer/ discussion moderator/ and time-keeper roles. Let the students who are presenting pick who ask questions, and let them provide their answers, too. And, let the students ask the questions. If you must, ask your ONE question last. Reward your students for asking good questions and make them aware that a reward is available beforehand (see our comments on assessment below). Jump in to encourage the more silent students to engage in the discussion; especially women and minority students. Step in quickly if a pair of presenters too quickly dismisses a valid question. And, react even more quickly to gender-bias or other effects on this type of dismissal. The basic mandate to the instructor is to create a safe classroom and facilitate the interaction among students to become a community of learners, which is what scientists are supposed to do.

COMMENTS ON THE QUESTIONS FOR FURTHER THOUGHT.

Comments on the Question. How exactly do stomata open and close?...
Comment for Instructors: ...this is right out of their textbook - beware of plagiarism.

Comments on the Question. Why do plants vary stomata number and not stomata size?
Comment for instructors: ...what you are looking for here is not necessarily a single correct answer but rather evidence of research and good ecological and scientific thinking. This is a complicated problem, and stomata morphology and function are active areas of research in plant physiology.

Comments on the Question. Why might it be adaptive for stomata to occur mostly... on the undersides of leaves?...
Comment for Instructors: ...part of this is right out of their textbook, but they will have to seek outside information on plants with unusual stomata configurations (such as some grasses, pond lillies, etc.) to complete their answers.

Comments on the Question. Photosynthetic and stomatal adaptations of cacti...
Comment for Instructors: ...part of this is right out of their textbook, but they will have to seek outside information on CAM plants such as cacti to complete their answers.

Comments on the Question. Leaf adaptations of mesic vs. xeric plants...
Comment for Instructors: ...part of this is right out of their textbook, but they will have to seek outside information to complete their answers.

Comments on the Questions. How might stomata density serve as a bioindicator... of climate change... and of paleoclimates...
Comment for Instructors: .....what you are looking for here is not necessarily a single correct answer but rather evidence of research and good ecological and scientific thinking. This is a complicated problem, and is an area of research in plant physiology. There are several very good references on stomata and climate change research in the Description: References and Links section.

Comments on the Question. In bryophytes, why might it be adaptive for only the sporophyte and not the gametophyte stage in the life cycle to possess stomata?
Comment for Instructors: .....what you are looking for here is not necessarily a single correct answer but rather evidence of research and good ecological and scientific thinking. This is a complicated problem, and frankly, we have not heard a definitive explanation of this, yet.

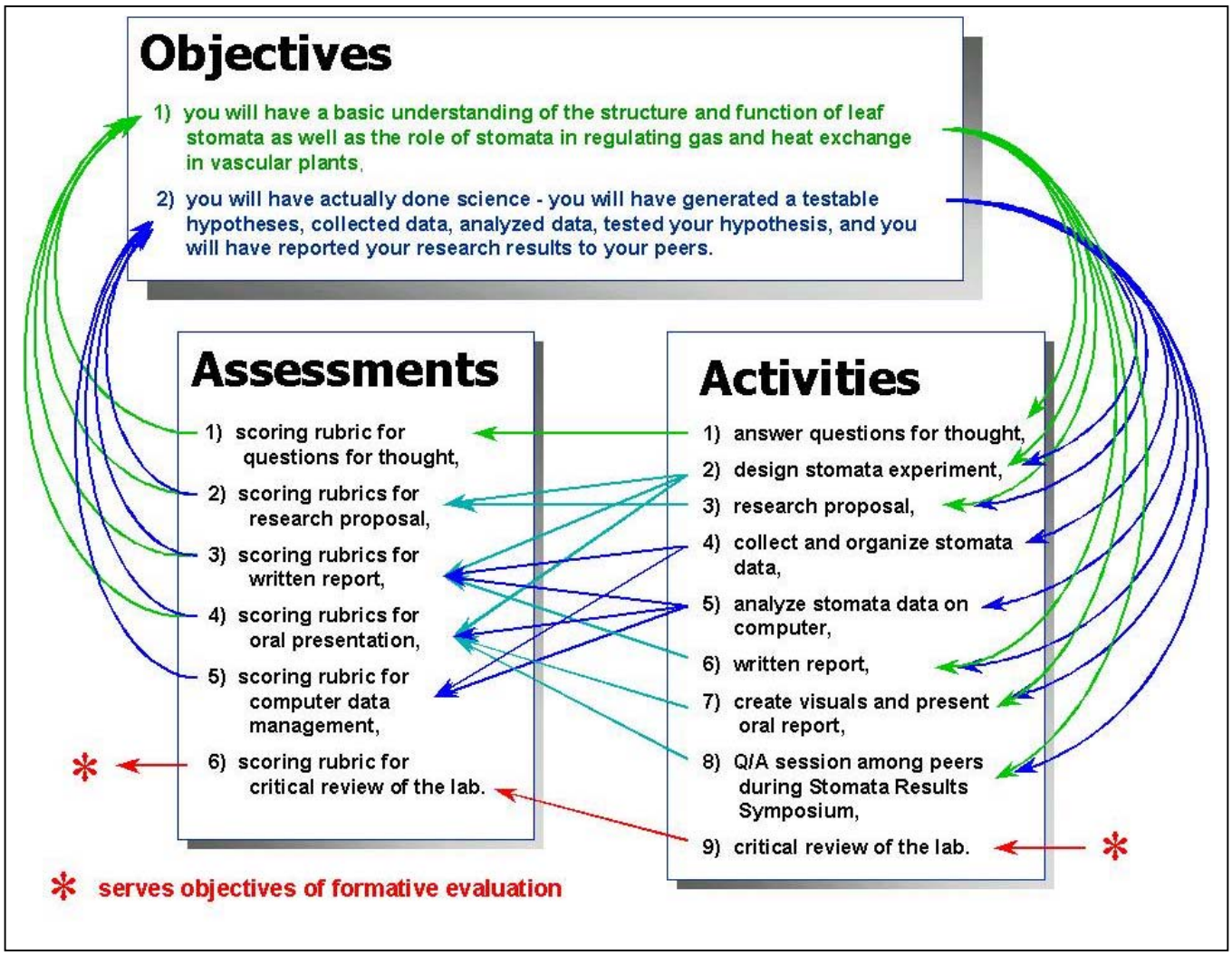
Comments on the Question. How might stomata density be affected along the urban - rural gradient...
Comment for Instructors: .....what you are looking for here is not necessarily a single correct answer but rather evidence of research and good ecological and scientific thinking.

Comments on the Question. What are some of the specific adaptations possessed by plant parasites to gain access to leaf tissue...
Comment for Instructors: .....what you are looking for here is not necessarily a single correct answer but rather evidence of research and good ecological and scientific thinking. This is an interesting ecological problem, and is of great economic importance to our agricultural ecosystems.

Comments on the Question. What role do stomata play in the solution to the problem of getting water up to the leaves...
Comment for Instructors: .....part of this is a classic problem right out of their textbook, but they will have to seek outside information to complete their answers.

ASSESSMENT OF STUDENT LEARNING OUTCOMES.

Below is a graphic to illustrate the relationships among the laboratory objectives, activities, and assessment instruments.



Below we explain how we have designed these assessment instruments for our classes. However, instructors should modify, omit, and/or add their own assessment instruments to meet the needs of your students. Keep in mind that:

- there must be a clear and unbroken network of links that map the objectives to the activities, to the assessment instruments, and then back to the objectives, and
- this map as well as all details of how assessment proceeds must be completely revealed to the students beforehand - students will attempt to perform only and exactly those tasks upon which they will be assessed.

Scoring Rubric for Questions for Thought.

We ask our students to submit written answers to any 4 of the questions. Students should use their texts or any other written references to answer these questions - but they must cite the complete and exact source of any text, web, or other outside material that they used. We strongly recommend that you read out loud to your students your course policy on plagiarism (which should be in your syllabus), and if you do not have one GET ONE!

Each citation of a research article or book should have: Author(s). Year. Title of paper. Journal. Volume: Pages. Each citation of an internet resource page should have: Author(s) if known. Specific Title of the Page. General Title of "Home" page/ Organization Name for the Site. Full "http" address. Date of Your Download.

Answers should be word processed, single spaced, 12 point, 1" margins, minimum 1/2 page in length, and in some cases including a well-documented Table or Figure. Our Scoring Rubric for Answers to Questions can be found in "Appendix 3: Scoring Rubrics."

Scoring Rubric for Stomata Research Proposals.

As described in the "Guidelines for Stomata Research Proposal" there are three parts to this assignment: Introduction, Methods, and Possible Results. In addition, students must generate an hypothetical graph of what their results would look like that would show an answer to their hypothesis about stomata variation.

Our Scoring Rubric for Stomata Research Proposals can be found in "Appendix 3: Scoring Rubrics."

Scoring Rubrics for Written Reports.

As is described in the "Guidelines for Written Reports", there are seven sections for reports: Abstract, Introduction, Methods, Results, Discussion, Literature Cited, and an Appendix. The maximum number of points for each section varies from 5-15 points, and 40 in total.

Our Scoring Rubrics for Written Reports for each section closely follow these guidelines and can be found in "Appendix 3: Scoring Rubrics."

In our experience, for the students' first drafts it is more consistent on our part to read and apply the scoring rubrics for all of the students' Introductions, all Methods, all Results, all Discussions, and then all Abstracts, rather than read each report all the way through and have to re-think out each rubric. Typically, we have about 12-16 students in each lab section, and two lab sections, which translates to 12-16 papers.

After we return the first drafts of our students' written reports, our students have two weeks to revise and re-submit (and many are sent to Widener's Writing Center for consultation sessions). The scoring rubrics above apply equally to their first drafts and revisions, and students base their revisions on their section scores and our miscellaneous written comments directly on their manuscripts. We also ask them to turn back in their original submission at the same time as their revision, which although it introduces some bias in our grading of their

revision, such bias is offset by our ability to compare their old and new versions and thereby quickly perceive their effort and allocation in their revision. In addition, we feel it is not entirely fair to them to “mark them down” for major new problems we discover in their revision that we should have caught in their first draft (however, this policy does not apply to spelling or grammatical errors that should have been caught by better proofreading).

Scoring Rubric for Oral Presentation.

As described in the “Guidelines for Oral Presentations", there are four sections for your reports: Introduction, Methods, Results and Specific Discussion, and General Discussion. The maximum number of points for each section is 10 points with a total of 40 points. Our Scoring Rubrics for Oral Presentations for each section closely follow these guidelines and can be found in “Appendix 3: Scoring Rubrics.”

In addition, during the Stomata Research Symposium, students should be rewarded for participation. This can be problematic if your expectations are not made crystal clear to then beforehand. We offer 10 points max for this facet of the activity, which represents 5% of the total grade. Our Scoring Rubrics for Symposium Participation can also be found in “Appendix 3: Scoring Rubrics.”

Scoring Rubric for Assessing Data Management.

As described in the “Guidelines for Data Management”, there are three computer files that constitute this part of the assignment: the manuscript, the presentation/graphics, and the spreadsheet data files. Our Scoring Rubric follows these “Guidelines” closely and can be found in “Appendix 3: Scoring Rubrics.”

Scoring Rubric for the Student Reflective Reviews of the Lab Activities.

As described in the "Guidelines for Reflective Reviews of Lab Activities", each student submits an individual review. Our Scoring Rubrics for Critical Reviews of the Lab follows these guidelines closely and can be found in “Appendix 3: Scoring Rubrics.”

Summary Table of Point Totals and Links to Detailed Scoring Rubrics:.

Rubric for Answers to Questions	40 points	individual
Rubric for Stomata Research Proposals	20 points	group
Scoring Rubric for Oral Presentations	40 points	group
Scoring Rubrics for Written Reports (1st version)	40 points	group
Scoring Rubrics for Written Reports (revision)	40 points	group
Scoring Rubric for Data Management	20 points	group
Scoring Rubric for Symposium Participation	10 points	individual
Scoring Rubric for Reflective Reviews of Lab Activities	20 points	individual
Total	230 points	75% group

…which is 20% of the total course grade.

In the extremely rare case that group participation is inequitable, or some other group cooperation issues arise, we will require individual submissions for the Written Report revision and Data Management. We has only been necessary a couple of times in the last 5 years.

FORMATIVE EVALUATION OF THIS EXPERIMENT.

In our "Guidelines for Reflective Reviews of Lab Activities" we describe an activity that collects information from students to evaluate the general design and specific events that occurred during this lab activity. This is a very useful assignment, and in our experience students enjoy being constructive participants in the design of their curriculum. Many comments are insightful and very helpful in our year-to-year revisions. We use a Scoring Rubric for Reflective Reviews of Lab Activities to grade these on a 20 point scale. Our Scoring Rubric follows these “Guidelines” closely and can be found in the Appendix of Scoring Rubrics:

Extensive notes on how to conduct formative evaluation are in the Teaching Resources sector of TIEE in an ESSAY ON EVALUATION.

TRANSLATING THE ACTIVITY TO OTHER SCALES.

This activity is HIGHLY TRANSFERABLE. It could be modified to run in any undergraduate introductory biology lab course (major or non-major) at any college or university. It also could run in introductory lab courses in botany, ecology, environmental science, or upper division courses in a variety of sub-disciplines. It could also be modified to run in biology lab courses in grades 8-12.

It would be difficult, however, to run this lab in a single three hour lab period. A key facet of this inquiry-based activity is the students' ownership of their question, sampling activity, analysis, interpretation, and presentation.

One aspect of this activity that also is HIGHLY TRANSFERABLE is the assessment scheme - please feel free to modify the scoring rubrics presented here for use in your laboratory activities.

CREDITS AND DISCLAIMERS

CREDITS FOR THIS EXPERIMENT:

This laboratory exercise was inspired by an article by Brian Drayton and Prassede Calabi "Long-term plant responses to environmental change: leaf stomata densities" that appeared in Hands On! Spring 1992. Vol. 15, Number 1, published by TERC (Cambridge, Mass, see www.terc.edu/handson), and by the laboratory activity "Responses by stomata on leaves to microenvironmental conditions" by Carol A. Brewer (1992) pages 67-75, in Tested studies for laboratory teaching. Volume 13. (C. A. Goldman, Editor). Proceedings of the 13th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 191 pages. (available at: http://www.zoo.utoronto.ca/able/volumes/vol-13/3-brewer/3-brewer.htm).

This submission was greatly improved by comments from anonymous reviewers, and by comments from Susan Will-Wolf and Reid Harris on the appendix on statistics.

GENERIC DISCLAIMER:

Adult supervision is recommended when performing this lab activity. We also recommend that common sense and proper safety precautions be followed by all participants. No responsibility is implied or taken by the contributing author, the editors of this Volume, nor anyone associated with maintaining the TIEE web site, nor by their academic employers, nor by the Ecological Society of America for anyone who sustains injuries as a result of using the materials or ideas, or performing the procedures put forth at the TIEE web site or in any printed materials that derive therefrom.

Lastly, we request that you return your students' and your comments on this activity to Susan Musante (smusante@aol.com), Managing Editor for TIEE, for posting at this site.