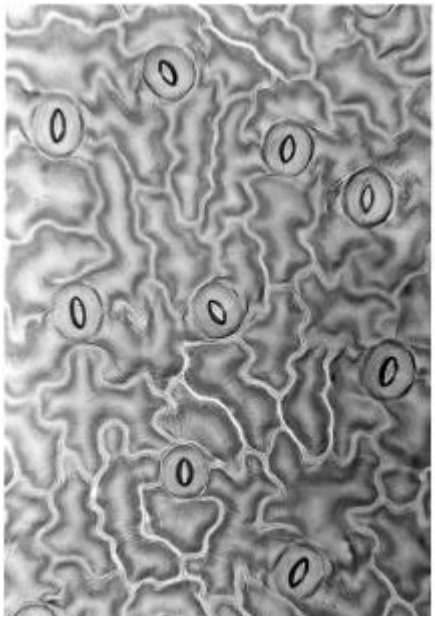
Experiments to Teach Ecology, Volume 2.

Teaching Issues and Experiments in Ecology (ESA - TIEE Web).

Environmental Correlates with Leaf Stomata Density



stomata impressions from a leaf underside made using clear nail polish viewed at 400x (photo by Marc Brodkin)

Contributed by:

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Contents:

Synopsis of the Lab Activity.

What Happens:

In this three week lab, you will work in pairs and generate and test an hypothesis of your choice about how leaf stomata density might vary under different environmental conditions. During the first week, you will learn about leaf design and the ecophysiological effects of variation in stomata density on leaf carbon, water, and heat budgets. You and your research partner will then design your own study to compare stomata density among leaves that differ in biophysical environment. Also during week 1, you will collect leaf samples and make impressions of their stomata using clear nail polish. During week 2, you will count stomata, make graphs, perform statistical analyses (t-tests) and generate a co-authored report on your findings. The lab period on week 3 is entirely devoted to a "Stomata Results Symposium" at which you will present your research results to your peers. There is a written research report and one cycle of revision.

Lab Objectives:

At the conclusion of this multiweek lab,

- ✧ you will have a basic understanding of the structure and function of leaf stomata as well as the role of stomata in regulating gas and heat exchange in vascular plants,
- ✧ you will have actually done science - you will have generated a testable hypotheses, collected data, analyzed data, tested your hypothesis, and you will have reported your research results to your 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:

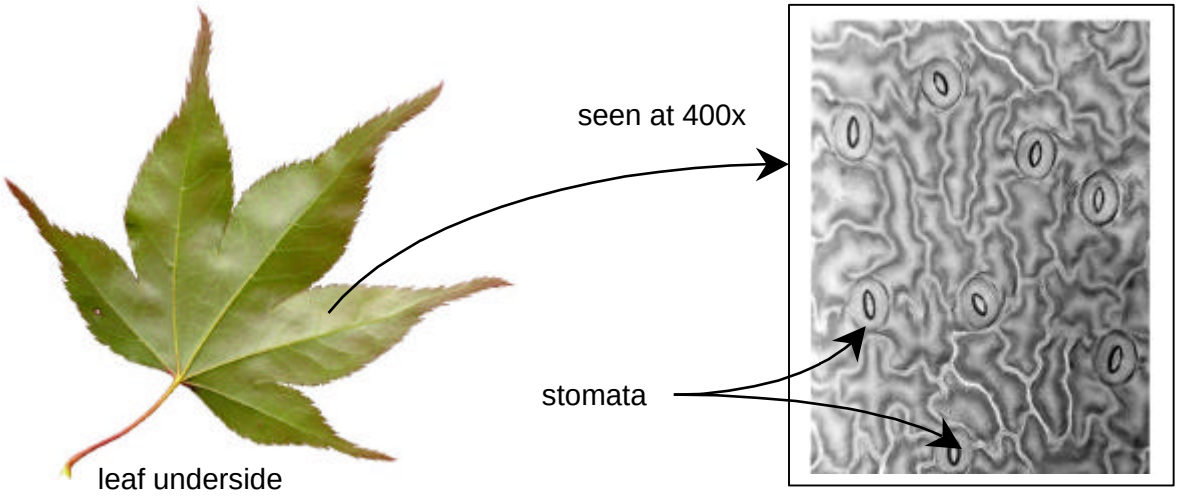
From this multiweek lab, you will submit...

- ➢ a one page research proposal co-authored with your research partner composed according to the guidelines below (due at the end of the first lab),
- ➢ answers to the questions for further thought contained in this handout (due in lab on the second week – please work alone on this part),
- ➢ co-authored stomata results report composed according to the guidelines and presented in class (due in lab on the third week, and then with one cycle of revision),
- ➢ clearly labeled hard and disk copies of your original data including the actual stomata impression slides, and disk copy of the visual aids created for their Oral Presentation (due in lab on the third week), and
- ➢ a critical review of the lab activity (due one week after the third lab – please work alone on this part).

Description of the Lab Activity.

Introduction.

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 below). 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.



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.

Overview of Data Collection and Analysis Methods.

Week 1.

During the first lab period, pick a research partner with whom you work on this project and:

- 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,
- decide on a place anywhere within about 10 minutes walking time where you intend to collect leaf samples in the environmental types of interest to test your hypothesis,
- bring your leaf samples back to lab and count their stomata densities (see "Detailed Procedure for Obtaining Stomata Impressions" below),
- 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 Density 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 Analyses of Stomata Data" 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"). Also on this date, your co-authored written report is due (see "Guidelines for Reports" below) as well as your disk copy of your data (see "Guidelines for Stomata Data and File Management on Disk"). Your individually written critical review of this multiweek lab activity is due the following week (see "Guidelines for Reviews of Lab Activities").

Detailed Procedure 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.

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^2." 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^2." {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}.

Guidelines for Your Stomata Density 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 "If our hypothesis were correct that under..... [fill in your choice] .... environmental conditions, then leaves should do better with increased stomata density.... 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 incorrect we would expect our data to show ..... see Figure 1..." Obviously, make sure that one can tell which pattern is which in your figure.

Guidelines for Analyses of Your Stomata Data.

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.

In Appendix 1 below is a detailed "Guidelines for Statistical Analysis" of your stomata data that includes a description of the t-test. Please read these pages carefully and consult with your instructor if things are still unclear.

Guidelines for 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 listed in Appendix 2 below. Please refer to "Appendix 2: Detailed Guidelines for Stomata Lab Written Reports."

Your oral presentations should also conform to a format for scientific presentations and the details you need to know are:

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

3). The principal differences between oral reports and written reports are that:
   a. oral reports do NOT start with the Abstract, they start with the Introduction,
   b. oral reports do NOT detail the methods as extensively,
   c. 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 Stomata Data and File Management on Disk

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 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, and (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.

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 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.

References and Links.

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Guidelines for Student Reviews of Lab Activities.

No lab activity is perfect and its participants, YOU, have important insights 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 in our experience, students often enjoy being more 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 the Stomata Lab activity (week 3), we want you to write a brief paragraph (wordprocessed, single spaced, 12 point, 1" margins, minimum 1/2 page in length), that conveys your most pressing concerns with the lab activity and exactly how the activity should be modified to improve it. For example, if a 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? What was unclear about the directions/ procedures, and what is needed to improve clarity? Was the assessment scheme understandable? Was the workload and grade for the individual/group parts fair? What specific lab objective(s) were not met, and what specific activity should be used instead that would better accomplish the lab objective(s)? To repeat, your comments must be constructive to be given credit.

Your critical/constructive review of the Stomata lab activity is due the week after the Stomata Lab Results Symposium. Clear, concise, and insightful reviews that demonstrate your reflection and constructive criticism of a lab activity will earn the highest credit.