

Physiological Dynamics in Animals and Plants - Laboratory 1 - Plant Growth at High Temperatures

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Introduction:

In the PDAP laboratory we hope to convey both the "what" and the "how" of animal and plant physiology. Instead of viewing this lab as just another set of facts to be learned, we hope that you will see it as an opportunity to practice some interesting experimental science. Over the first few weeks of the lab experience we plan to introduce you to some intriguing physiological phenomena and allow you to design and perform some experiments to characterize those phenomena. Later in the course you will choose your own experimental system *and* design experiments to test a hypothesis that you have formulated about that system.

Today's physiological phenomenon: Plant growth responses at higher temperatures

There are two ways that plants grow: 1) by elongating existing cells in their roots and stems; and 2) by cell division at the tips of roots and stems. The plant growth hormone auxin is required for both. Recent studies suggest that at least some types of plant cells may become insensitive to this important growth hormone at higher temperatures so that the cells are no longer able to grow or divide. There are also studies to indicate that heat disrupts other aspects of plant growth and development that are hormone-mediated. With global climate change looming there is concern that heat may slow, prevent, or alter the growth and reproduction of certain plants including some important crop plants.

Our **goals** for this lab are to: a) formulate a testable biological hypothesis about the effects of heat on plant growth and development; b) test the hypothesis experimentally; and c) analyze and interpret the data; and d) write the hypothesis, methods, results, and conclusions. You will work in teams to do a-c; each of you will write your own report.

Experimental:

This week, you will:

1. Surface-sterilize seeds of *Arabidopsis thaliana*, thale cress, and plant them on sterile agar containing a mix of nutrients that plants need to grow. You will incubate these plants under continuous white fluorescent light for one to two weeks at room temperature.
2. Go to the NCBI website and use pubmed (<http://www.ncbi.nlm.nih.gov/pubmed>) to find a recent (2005-2009) primary journal article containing the key words "heat", "auxin", and "plant". You will read this article, decide as a group what growth characteristic(s) you wish to measure, and make a hypothesis about how heat might be expected to affect this/these growth characteristic(s).

In weeks two through four you will:

1. Measure the growth characteristic(s) of interest to get a baseline value;
2. Transfer some plants to a cooler lighted incubator and others to a warmer lighted incubator;
3. Re-measure the same growth characteristic(s) that you measured in (1) after an additional week to ten days at each of the two growth temperatures.

After your seeds have been planted while your plants are growing, work with your lab partners to design your experiment.

Each experiment begins with a **hypothesis** - a statement of the idea that you hope to test. You then design an **experimental protocol (method)** to test the hypothesis. The protocol must be designed with **control experiments** that will eliminate all other plausible hypotheses as explanations for the phenomenon you are investigating. Your experiment must be technically feasible. After you have collected your data, you must determine the best way to present your **results** (text, tables, graphs). After looking at the data, you will draw a reasoned **conclusion** about whether they support your hypothesis or not.

By week five turn in a two-page report (excluding figures, graphs, tables; no more than 2 pages of text!!)

Organize the report as follows -

Hypothesis: State the hypothesis that you tested, then briefly describe the rationale that led you to formulate it, citing the primary journal article that you found in pubmed.

Experimental protocol: Describe exactly what you did in enough detail that another scientist could reproduce it. (This section is often called Materials and Methods in a scientific journal article)

Results: In short sentences, briefly describe your results with text, referencing any figures, tables, graphs, etc. Do not repeat the same data in text and then figures, and then tables, and then graphs, etc.

Conclusion(s): State your conclusion(s) - be sure to state whether the data support or do not support you original hypothesis. **DO NOT** use the word "prove"; scientists seldom, if ever, prove things. Leave room for the possibility that someone else might eventually think of experiments that would cast doubt, if not negate, your conclusions.

Some details of methods for your report:

Sterilizing and germinating seeds: *Arabidopsis thaliana* (thale cress) seeds will be germinated and plants will be cultured under controlled conditions (aseptically on a well-defined nutrient agar under continuous fluorescent light at room temperature). *Arabidopsis* seeds will be surface-sterilized in microcentrifuge tubes for 10 min in an

aqueous solution containing 10% bleach; 10% ethanol; and 0.01% Tween 20, a nonionic detergent. Seeds will be collected by centrifuging for 1 min at top speed in a microcentrifuge. The supernatant will be discarded. Seeds will be re-suspended in sterile water and collected again by centrifugation. The latter step will be repeated four more times. The final time that seeds are resuspended, 100 microliters of suspended seeds will be pipetted onto the surface of nutrient agar in baby food jars. Jars will be sealed with vented caps. All of these operations will be performed in a laminar flow hood in which the air inside the hood is sterilized by passing it through a high efficiency particulate air (HEPA) filter. Dr. Tallman will divide the class into groups and schedule each group for a time to use the laminar flow hood. He will teach the first group; then each group will teach the next group. Seeds will be incubated under continuous white fluorescent light (50 micromoles per square meter per second of photosynthetically-active radiation [PAR]) in Olin 101 until seedlings are large enough for experiments, usually a week to ten days after planting.

Other details of growth conditions, etc. will be provided in lab, so please take good notes.