What's a lab *FOR?* Working Together to Optimize the Laboratory Experience and Teaching Space

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Abstract

The laboratory is a learning environment in which students must not only refine their skills, but must also do so in a safe manner. The mental skills of dealing with uncertainty and experimental design should be challenged at the same time that physical laboratory skills are learned. In addition, the space in which students work on their laboratory skills is of the utmost importance for students as it determines their safety while being challenged to become competent scientists. This ABLE session provided a round table for a dialogue about planning and organizing both laboratory experiences and teaching space. Participants collaborated to come up with the skills and abilities that we think laboratories should be designed to build. A hands-on demonstration showed how an open-ended investigative lab can be constructed using simple equipment. Following this several laboratory designs submitted by colleagues were showcased. Ideas about what is good in these laboratory designs and what could be improved were investigated.

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Introduction

We all know that the study of biology, or any other science, involves more than assimilating factual information. It also involves learning how to effectively use that information for problem solving, posing hypotheses, conducting experiments, and interpreting experimental results. Given this, if we want our students to understand what science is, we need to provide them with both conceptual knowledge and practice in using that knowledge. That is, we need to give them opportunities to actively practice the process of science. The laboratories associated with biology courses seem an obvious place for providing this practice.

The laboratory is an environment in which students can actually "do science". It's usually the most expensive part of science course delivery, but is worth the effort in that it builds skills, fosters a professional attitude, and gives students an opportunity to "test drive" their future career. However, for this to be true, we need to design our laboratories such that they engage our students in the process of science.

While this is easy to say, exactly how we do we do this? How do we engage our students in the process of science? How do we do this in the confines of a student laboratory? How much does this cost? Are there models that we can use in developing these types of experiences? Can we do this in our current laboratory space? If we could remodel our laboratory space, what designs facilitate these experiences? Since funding for lab renovations is difficult to come by, what can we learn from a variety of existing designs?

In other words, we need models not only for the development of the lab experiences themselves, but also for the laboratory space or design. Not only are laboratory experiences challenging to develop and staff, but they also require an appropriate physical space which can take a substantial amount of capital to build. Colleagues who are lucky enough to have input in strategizing the construction of new teaching laboratory space usually can relate war stories and show battle scars from their dialogue with administrators and architects. Details we take for granted, such as placement of electrical outlets and safety equipment loom large in the planning.

This workshop provided a round table for a dialogue about planning and organizing both laboratory experiences and teaching space. Participants collaborated to come up with the skills and abilities that we think laboratories should be designed to build, and compared them with results Jean Heitz has accumulated during her career as an educator and laboratory coordinator. There was a hands-on demonstration showing how an open-ended investigative lab can be constructed using simple equipment. We finished by showcasing several laboratory designs submitted by colleagues. Nancy McInerney related how she went about renovating and retrofitting laboratories at Red Deer College. Todd Nickle spoke briefly about the construction of a new laboratory building at Mount Royal University. Ideas about what is good in these laboratory designs and what could be improved were investigated. These Proceedings summarize the session.

An Active Learning Environment - Developing science experiences

The purpose of ABLE is to promote excellent science education through a student-centered, active learning environment. If we focus on the process of science in the laboratory, we can provide our students with opportunities to learn how to:

- Apply their conceptual knowledge as they investigate novel questions or problems.
- Devise their own methods or protocols
- Execute their proposed experiments
- Analyze and interpret the data they collect
- Develop logical arguments and other critical thinking skills
- Report the results in both written and oral scientific format

In addition, we can help our students develop their abilities to work well in teams. When students work in teams they can explore more complex and meaningful, "real-life" questions or problems. Working in teams also develops students' abilities to collaborate and work effectively with others.

What outcomes can - or should - the laboratory experience produce?

Educators and students have been surveyed about what skills scientists use regularly. Interestingly, the outcomes Jean Heitz collected from educators and students match each other very closely – including at the 2009 ABLE meeting! As shown in Table 1, these also align with Essential Learning Outcomes published by the Association of American Colleges and Universities (2007).

But how can we facilitate students achieving these outcomes? This is no small task. In our courses, we try to challenge our students without pushing them so hard that they give up. We know when they are pushed too hard, students can cease their explorations and wait for the instructor to demonstrate what needs to be done next. We also have very real budgetary limits. As a result, when developing a lab exercise or when redesigning the physical laboratory space, we have to live within these. While these are all real limitations, none of them dictates the types of learning outcomes we want for our students. Instead of walking students through techniques in isolation, why not let them grapple with open-ended questions? Make the lab experience mirror real research. We already have a wealth of open-ended exercises for students to work through – this is the lifeblood of ABLE.

In our minds, the laboratory environment is a place to bring our students to terms with the fact that ninety percent of what scientists do, they do in their heads. There's no magic formula for designing an investigation of the natural world. An observer has to decide what s/he thinks is happening and then devise a way to see if their understanding correlates with the reality (*i.e.* test a hypothesis!). Work with what you have, but be creative. A laboratory module can certainly last for more than one session. Give the students time to reflect. You can introduce the students to the problem, techniques, and the tools in week one, and give them time to develop a proposal for their study. During the second week, students conduct their research. In a final session, students can analyze their results and communicate them.

Table 1. Typical responses collected from stakeholders in science education when asked about the skills andabilities used on a regular basis during a scientific career. Faculty answered based on the skills they actuallyuse. Students answered based on what skills and abilities they expected to use regularly. These are comparedwith Essential Learning Outcomes, published by Liberal Education and America's Promise (LEAP)

Educators and Students	LEAP Essential Learning	
	Outcomes	
 Problem solving skills Communication skills (both written and oral) People skills, e.g. ability to work well in groups Ability to think critically Organizational skills Ability to learn from one's mistakes and a willingness to continue learning Ability to be flexible, to think on your feet Having a good base knowledge of the material 	 Knowledge of Human Cultures and the Physical and Natural World Intellectual and Practical Skills Inquiry and analysis Critical and creative thinking Written and oral communication Quantitative literacy Information literacy Teamwork and problem solving Personal & Social Responsibility Integrative Learning Synthesis and advanced accomplishment across general and specialized studies Demonstrated through the application of knowledge, skills, and responsibilities to new settings and complex problems 	

With time to think and reflect, students can learn more. Furthermore, the lessons can be deliberate. If you want students to learn, the instructor must be clear about *what* the student should learn from the process: the types of scientific processes, how to think and organize ideas, and what methods they should be picking up.

Another skill we can help our students with is not as obvious: how to deal with uncertainty. This is something established scientists expect and must be comfortable with whether they consciously realize it or not. Students often don't understand that the true nature of science is to work with uncertainty. They've often been exposed to a curriculum with predetermined outcomes, and have come

to the conclusion that science is a clearly defined set of steps to come up with The Answer. They don't view science as a process, while we scientists view the laboratory as a place to embrace uncertainty and make sense of it.

The laboratory is also a place where students learn the skill of dealing with their mistakes. For this reason, the exercises must **not** be constructed with the specific outcome as the most important goal. Focus instead on what the students have learned. Try to identify if and when the students have made a mistake and how they dealt with it. And be sure there's an opportunity for them to make those mistakes! Providing all the steps to solving the problem and creating a finely-tuned exercise with exquisite detail removes the chance that students will develop the ability to think and do for themselves.

The real learning is up to the individual. Much of this will probably occur outside the classroom. However, the laboratory environment can give students practice in different ways of thinking, and they can carry this out the door. They should learn that science is a process. It is simply a tool that we use to deal with uncertainty. They should also understand that there are three simple questions that they should use both in our in-class labs and when doing science as a professional:

- What are we doing?
- Why are we doing it?
- How are we doing it?

These are the basis for all of scientific investigation. As such, they should be the object of our teaching. ABLE is a resource for science educators, and provides good, open-ended research projects that can serve as the basis for student research. Appendix A of this paper includes one such exercise: a simple wet lab regarding gravitropism and the hypocotyl which can be done in almost any kind of room.

Important things to consider when developing a laboratory focusing on the process of science

- *Cost.* For most of us it would be prohibitively expensive to completely discard our current laboratory experiences. Instead of doing that we will consider how the labs we do at present could be converted into more open-ended, process-oriented labs.
- *Intent.* What is it that we want our students to learn? We all recognize that real learning is the responsibility of the individual and that the majority of this occurs outside of the classroom. However, what goes on in the classroom and what we require of our students should set the stage for and give students practice in the learning we want them to achieve.

- *Activities.* The laboratory is an environment for training students in Good Laboratory Practice and also in how to perform scientific investigations. Science is not a collection of facts, but instead is a way of investigating the natural world. When we challenge students to create their own hypotheses and strategies to investigate them, our students also learn that:
 - Not all problems in life are clearly defined and there will always be some level of uncertainty.
 - Most research problems are complex and no single experiment is likely to solve an authentic problem.
 - The value of any experiment is determined by how carefully the researcher develops the methodology and controls, and by the quality of his/her data collection, analysis, and interpretation.
 - Research costs both time and money. Therefore, not all experiments are designed to be definitive. Some are instead set up "quick and dirty" to provide insight into worthwhile next steps.
 - An experiment cannot prove anything it can only disprove or lend support to an idea. As a result, research experiments are most frequently designed to eliminate possibilities.
 - A good experiment usually raises a number of questions that will lead to further experimentation.
 - All experiments succeed; however, they may not give us the results we want. As Thomas Edison once said: "I have not failed. I've just found 10,000 ways that don't work."

In addition to all of this, our students should gain more practice in how to effectively problem solve in groups, how to analyze data, and how to communicate their understanding effectively in written and oral formats.

An Active Learning Environment – Developing the physical space

In a process of science setting, many of the laboratory opportunities that we present can involve non-traditional techniques and unusual station setup. Sometimes group work requires that tables be moved, or there might be a lot of moving around of students during, say, a demonstration of how a contagious disease is dispersed. To be effective, the physical design of the laboratory space must accommodate these.

We usually have a specialized physical space for science students – the laboratory. This is a dedicated, expensive domain in which safety must be balanced with both functionality and comfort. Solitary workstations might need to be rearranged or repurposed for group work, e.g. by putting the workstations/tables on wheels. But if you're going to have sensitive equipment like microscopes on a table, do you really want that table on wheels? Are there types of tables on wheels that can address such concerns? Laboratory layouts are quite diverse, and seldom suit every user perfectly. How, then, should one approach the development of the laboratory room?

The stakeholders

Whether building from scratch or renovating, it's important to find a way to get the *end users* involved right from the beginning. The instructors, technicians and even the students have good ideas about how the laboratory "works", and can walk through in their minds how a variety of arrangements would work. For example because food isn't allowed in the laboratory, students have mentioned that having unassigned lockers in the hallway would be helpful as they can store their lunch, an idea that sometimes doesn't occur to the instructors or architects.

Get one or two representatives to be on the building committee. These representatives should be included in discussions with architects and engineers, and at these meetings should be prepared to state what they want that enhances teaching and learning. These ideas will need to be adaptable to new technologies and equipment. It's hard to plan 15-20 years in the future, but you'll certainly be expected to use the space for that long! When designing the teaching space, ask for the best, but expect to have items cut as costs escalate. Prioritize these items so you know what you can live without. The representatives will work with the builders to communicate these needs on a regular basis – both to the builders and the end users.

Equipment which might be relevant to your teaching space

It's sometimes surprising how specific the architects are with respect to the details of your work area. The exact spacing of power outlets can come up during discussions. Fume hoods can fail to work safely in areas of high traffic or near windows. As a result, their positioning in labs is critical. Is there going to be a place to store those ubiquitous backpacks? What about a coat rack? All of this must go into the footprint of the plans before construction is even to begin.

Some items that you might bring to the discussion with the building committee are:

- white boards or blackboards (y/n, how many)
- types of ceiling lights: fluorescent bars and/or pot lights with slide adjustment
- data projector(s) and screen(s) locations
- wireless or wired internet or both
- number and locations of light switches and electrical outlets
- types, sizes, numbers and locations of sinks
- distilled water, compressed air, valves for other compressed gases and vacuum taps
- number of each type of tap per room
- arrangement of tables or benches
- presence/absence of pull out writing surfaces on fixed benches
- availability of prep/set up space in the lab room
- proximity of the labs to prep room for technicians
- types of ventilation required
- availability of biocontainment or sterile work areas (such as a laminar air flow)
- special requirements such as power, sound proofing, light or lack of light, vibration control or special ventilation needs

Efficiencies and savings

Whether building new or renovating, the equipment can come from a variety of sources – even from "previously loved" sources (*i.e.* used equipment). Consider what can be recycled from older parts of the institution and, if renovating, what should be discarded. Furthermore, who is to decide this, and how will the decision be reached? Most everyone reading this has discovered "pots of gold" in the laboratory supply area –old models or teaching materials that might have gone into the garbage but have gained new life when cleaned up. Even the manager of the laboratory might not know every use for every single inventory item. Many items have been discarded that could have been useful but for the lack of someone noticing their trip to the landfill.

Refinishing older furnishings may be feasible, but you have to be sure that there's enough time for this and that you can find skilled and accessible trades people. In Alberta, an oil-producing region, the trades have historically been in tight supply. Recently, however, economic adjustments have provided an opportunity for institutions to get faster turnaround on their projects: a new resource for us to exploit. Some items are very durable and easily re-used. Stainless steel sinks can be cleaned up for a fraction of the price of purchasing new. Wood cabinets are very amenable to cleaning up and reinstallation. When choosing furnishings, you can choose to use a local manufacturer or a national supplier. There's a trade-off: local businesses can be more flexible with what they deliver, and can modify on site, but the larger suppliers might provide a more uniform product.

As for renovating laboratory rooms, you need to consider the age of the space. Retrofitting may no longer be economical if building codes have changed. Wiring, ventilation, waste water disposal / collection all need to be considered. Asbestos containment can add significantly to the time required to dismantle the space before renovations begin. Old pipes may have broken or collapsed. All of these add up to extra, sometimes unexpected, costs.

It's sometimes possible to share equipment or renovation costs with other departments. Centrally located prep and/or equipment rooms can allow broader use of the equipment in a variety of areas. Steam and water systems in these central rooms can service multiple laboratories. You might be able to contact a supplier to obtain "gently used" equipment that has been returned because although it works perfectly, it did not suit a customer's needs. Also, ask about and apply for manufacturer educational grants when possible.

A webpage (http://www2.mtroyal.ca/~tnickle/ABLE09-LAB) provides an archive that links to videos showing some of the authors' teaching spaces as well as photographs of other teaching laboratory spaces.

Important things to consider when developing a laboratory's physical design

- *Cost.* Obviously, there will be limits as to what an institution can afford to put into place. The cost of computers is coming down, but there's always a "new and more expensive" model on the horizon that could have a good impact on your teaching strategy. The larger the room, the more it will cost, not only in construction but also in heating and maintenance. Paying more to get extra electrical outlets may be worthwhile, as retrofitting can be cost-prohibitive. Do you need vacuum lines, or will an aspirator suffice? Many of us have had to make sacrifices that we've "learned to live with". Hopefully good planning will result in less "buyer's remorse"!
- *Intent.* It's not possible to predict all the activities that will go on in a teaching laboratory. Windows can be useful to provide light for growing plants, but they also can restrict shelving space. If the laboratory exercises are something students can take ownership of, perhaps they'll require movement of tables and the ability to leave apparatus set up for more than a week at a time. Will Honours students have access to the area for their projects? Some labs are strategically positioned next to classrooms so the students can be provided a comprehensive introduction, or called back for organizational instructions during the exercise.
- *Multifunctionality.* Smaller universities and colleges might not have the enrolment numbers to support labs dedicated to only a single course or field of study. Safety issues (introduced below) may preclude the use of microorganisms in all but particular spaces. As a result, you may not be able to share or use that area for other labs!
- *Storage.* What kinds of equipment will likely be needed? Can it stay in the teaching lab? Will it be shared? How sensitive is it to relocation? Shelves are nice, but tend toward untidiness. Students will have to have a secure place to put backpacks and books as well. Day lockers outside of the lab can be helpful to prevent clutter near exits. Having a study area nearby where food can be consumed outside of the laboratory area is appreciated by students, perhaps avoiding the need of a "food table" in the hall outside of the lab where students will put their coffee and sandwiches. At Mount Royal University, we faculty were surprised by a request for a list of what chemicals we would store in a proposed laboratory construction project. Industry rules require that the builder takes into account a fairly detailed inventory list prior even to breaking ground. We still don't know what projects we'll give to the students, but were required to provide a list of chemicals for those unknown labs!
- *Common equipment areas.* These will have to be conveniently placed, and equipment will have to be stored in a safe way. Smaller faculty members can't reach up as high, for instance, and heavy items placed in higher locations can be dangerous to remove and replace. Delicate instruments must of course be easily accessed and not subject to long, dangerous trips. Items that require routine maintenance must also be accessible (out of sight, out of mind!).
- **Prep rooms.** Where the instructional assistants will have room to prepare course materials and take care of disposal matters a lot to the employee, but also has implications for the safety of everyone in the general area. You wouldn't want to travel a long distance in public corridors

with chemical or biohazardous materials. The prep area will have to adequately deal with issues such as safety, access, storage, waste treatment, water treatment, chemical storage, biosafety, *etc.*

- **Delivery.** Will the instructor be a "guide at the side" or a "sage on the stage"? Will students have to access the lab out-of-class in order to make measurements or perform activities? Is a resource person available in a Study Area? Often our laboratory instructors will need to demonstrate how to use a piece of equipment. If this is the case, there needs to be an instructor bench with a lot of room around it for the students to gather. Line-of-sight can be affected and detailed manipulations hard to see. Will there be a video projector and computer available? If so, a webcam could be used to display the knobs the instructor uses, or proper placement of sensors. The instructor will also need to be able to circulate within the lab space during the activity to check on the progress of students and provide advice.
- *Technology.* The use of simulations and PowerPoint presentations are becoming commonplace. While these can be helpful in getting students to understand some of the discipline concepts, hands-on opportunities need to be introduced as well. In many cases, simple apparatuses can be constructed from available materials. The benefit of going low-tech is that there's no imposition of a "black box" that technology can present. Instead of using an oxygen sensor to look at photosynthesis, sometimes showing how leaf disks bob in a column of water, or how a bubble of air moves in a bent pipette can demonstrate the principle. (One of this workshop's presentations demonstrated how to use camera film containers to address a simple plant growth question.)
- *Activities.* The laboratory is an environment for training students in Good Laboratory Practice and to also perform scientific investigations. Science is not a collection of facts, but instead is a way of investigating the natural world. When we challenge students to create their own hypotheses and strategies to investigate them, flexibility of assets in the learning environment is important. The students might need to reposition desks or have access to dark cabinets to create their experiments. Encouraging creative thought and then dashing the ideas because of restrictions of the work environment is disheartening to both instructor and student.
- *Safety.* Experienced laboratory instructors will first focus on the access and maintenance of safety stations. Eye washes, fire extinguishers, and safety showers are standard fare, but strange omissions, like drains under the showers, or distribution of the stations to different areas of the lab, can reduce their efficacy. Having a "panic corner" takes the guesswork out of where to go to access safety equipment. In today's climate, access to a telephone and the response time of security to an incident may be important to consider. Secondary exits in the case of fire are prudent to anticipate. Placement of fume hoods and laminar air flows needs to take into account the position of doors, air handlers, and windows in order to work effectively.
- *Future growth.* You'll probably be called back in the future to do the planning all again! Can you put in good design features to accommodate new applications and technologies? New courses? New labs?

• **Durability and appearance.** What colour paint on the walls? What kinds and colours of tiles? Solid? Patterns? The selection of flooring, cabinets, countertops, sinks, and chairs should be low maintenance and look good over years of student abuse! Maybe you should think ahead as to how you'll treat a stain on the wall? Remember, you can bleach white surfaces!

Literature Cited

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APPENDIX A

Student Outline

Gravitropism in hypocotyls: developing and testing a hypothesis

When working with a new or unknown system or organism, scientists usually begin by gathering baseline data. The initial exercises in this lab are designed to provide you with some baseline data on the behavior of the hypocotyl of *Brassica oleraceae* (broccoli). These initial observations will also be useful when you begin developing hypotheses and the rationale for these. Such experiments can also help you determine if you have any biases or "preconceived" notions of how the system or organism "should act". Using these preliminary observations you will propose and test a hypothesis concerning the mechanism(s) affecting the gravitropic response of the hypocotyl in *Brassica*.

As you make your observations and conduct your experiments, keep in mind that the hypocotyl is neither a stem nor a root. It is the transition region between the stem and the root in the developing plant. Therefore, it can respond differently than either stem or root to various stimuli. You can think of the esophagus, stomach and small intestine as an analogous system. All of these are connected to each other. However, each is distinct in its structure, function and responses to stimuli. The same is true of root, hypocotyl, stem and cotyledon.

Week 1

Before coming to class

- 1. Read the relevant pages in your textbook.
- 2. Answer these Pre-Lab Questions.
 - A. List three factors (parts or substances found in the plant) which are thought to be involved in the gravitropic or phototropic responses.

		Effect on:	
	Factors:	Gravitropism	Phototropism
А			
В			
С			

B. Where and how is each of these thought to affect gravitropism or phototropism?

	Gravitropism		Phototropism		
	Location:	Effect:	Location:	Effect:	
А					
В					
С					

3. Select one of the factors you listed above (or any other you think is reasonable) and develop a hypothesis concerning the mechanisms involved in the gravitropic response of the hypocotyl in Brassica. (Note: We are asking you to look at only one factor (or a very few closely related factors) to determine what effect it may have on gravitropism in the hypocotyl. We are not asking you to determine all of the factors that may affect gravitropism in the hypocotyl of Brassica.)

Write the hypothesis you plan to test here and in your lab notebook. Be sure to include a brief rationale for your hypothesis.

What kinds of equipment or supplies will you need to conduct an experiment to test the 4 hypothesis you propose in 3 above?

LEARNING THE SYSTEM

At the beginning of the lab period your instructor will:

A. *Give you a brief explanation* of the growth pattern of *Brassica*.

For your reference, Figure 1 illustrates the early life cycle stages of *Brassica* and identifies the various parts of the early plant.



Figure 1: early life cycle stages of Brassica.

B. Provide you with information on the test canisters that were set up prior to lab.

To set up the demonstration canisters strips of filter paper are cut to size (approx. 3/8" x 1 and 3/4"). A single strip of wet filter paper is oriented vertically along one side of the film canister. The wet filter paper sticks to the side of the canister because of the adhesion and cohesion properties of the water. Approximately 2 ml of water is added to the bottom of the film canister (enough to make a dime-sized drop).

A Brassica seedling is cut and its cotyledons are moistened and placed against the wet filter paper near the middle of the strip. As with the filter paper, the adhesion and cohesion properties of water will hold the cotyledons to the filter paper. The contact between water in filter paper and that on the cotyledons can be increased by gently pressing the cotyledons onto the paper. The canister is then capped tightly.

It takes from 3 to 6 hours to get a significant response from seedlings oriented in this direction. The demos for the lab are generally set up the night before or early in the morning before the lab.

Seedlings can survive in canisters up to 3 days (given sufficient water). By about the 3rd day, they will start to regenerate roots.

Next, your instructor will ask you to:

C. *Come to a group consensus* on how you feel the hypocotyl will respond in the first demonstration experiment (see Figure 2).

Proposed Results:



Figure 2: The hypocotyl initial setup (left) and predictions as to the outcome after 3-6 hours in the dark.

In addition, s/he will ask you to provide a rationale or reason for your proposed answer.

Two members of your group (selected by the instructor) will report your findings. One member will draw the expected response on the blackboard. After all groups have drawn their expected responses, another member of your group will explain the rationale behind your proposed result.

After all groups have explained their hypothesis and rationale, you will observe the response of the hypocotyls in the demonstration experimental chambers that were set up prior to lab.

Have a member of your group draw your observed response (use different colored chalk) on your original blackboard drawing. Make this as exact a representation as possible.

Are all responses the same? If different, how do they differ? What might account for the results observed and any variation in the results?

D. *Develop a hypothesis* for the orientation of the various hypocotyls in the "crucifer cross" demonstration (see Figure 3), also set up previously.

In other words, as a group, you will need to develop a hypothesis for how each of the 3-4 hypocotyls arranged in the canister will respond.

W

Figure 3: Experiment 2 setup with four hypocotyls arranged at the top (N), bottom (S) and both sides (E and W) of a film canister set on its side. The canister can be stabilized by taping an extra cap to the base so it won't roll (not shown).

Proceed as in C above. Then observe the response(s) in the "crucifer cross" chambers.

E. Determine how you would quantify the response

What measurements need to be taken to show a difference (or similarity) in response among the plants in the canisters? What kind of statistics might you perform to determine whether any difference seen is significant?

Generate a hypothesis and rationale

Within your group, compare your original hypotheses concerning the mechanisms involved in gravitropism of the hypocotyl. Choose one factor and develop a group hypothesis for the gravitropic response. For example, you could develop a hypothesis about how presence or absence of starch granules in the hypocotyl would affect the gravitropic response. Alternatively, you may choose to investigate another type of question using the hypocotyl system. Your design should take into account the limited available equipment and chemicals. Include adequate controls.

Your preliminary proposals should include:

- 1. Introduction (brief) State the purpose or hypothesis of the experiment and the rationale for it.
- 2. Materials and Methods State what equipment will be needed and explain the experimental design. This section should include a discussion of the controls to be used.
- 3. Results State what type(s) of data will be collected and how the data will be analyzed once collected. Include a discussion of the responses you would expect to see if your hypothesis is supported.

Week 2

CONDUCT YOUR EXPERIMENT

Groups will reconvene to review the comments received on their preliminary proposal. 15 to 20 min.

- 1. Each group will revise their experimental design, as needed, and each student will write the revised design in his/her lab notebook. (15 to 20 min).
- 2. Each group can then execute the experiment according to the design developed. (30 to 45 min to set up experimental canisters).

The experiments may continue over the next 3 to 24 hours in lab as needed.

Crucifer Cross Arrangement

Week 3

COMMUNICATE YOUR FINDINGS

A. Groups will reconvene to analyze experimental data and develop a research presentation (60 to 75 min). This could be in the form of a paper, poster or power point presentation. Your instructor will let you know which format to use.

The questions on the worksheet (following pages) are designed to guide preparation of your presentation. Unless otherwise indicated, your instructor will collect both your presentation and your worksheet at the end of the lab period.

B. During the second half of the lab, the groups will each present their draft hypothesis, experimental design, data and analysis to each other for discussion and critique. Approx. 5-10 min/group. All students in the group should be ready to explain the entire project. The instructor will call on members of each group randomly to explain individual parts of the project.

KEEP COMPLETE NOTES ON ALL OF THE STUDENT PRESENTATIONS. Unless otherwise indicated, a quiz on this lab module will be given next week. The quiz will be open book. Each student will be responsible for his/her own project and for the information provided by other groups during their oral presentations.

LAB #_____ NAMES:

Worksheet for Gravitropism and the Hypocotyl Lab

Fill this out prior to developing your research presentation. This information should be included in your final presentation and this worksheet should be attached to the final presentation.

INTRODUCTION - Be sure to cite references as appropriate.

- 1. What was the overall or larger goal of the research?
- 2. What was the specific goal (smaller goal) of your experiment?
- 3. Why did you choose this specific goal, i.e. what is your rationale for doing this specific project/research?

MATERIALS AND METHODS - In addition to descriptions, use illustrations/drawings, *etc.* to help explain your experimental setup.

4. What treatments/tests did you perform?

- 5. What was each treatment designed to test/discover?
- 6. How many specimens/organisms did you use per treatment or test and how did you control for variability among them?
- 7. What did you measure to determine if a treatment had an effect and how did you measure it?
- 8. What types of comparisons did you make between treatments and controls?

10. How do the results compare among treatments and between treatments and controls?

11. Are any of the differences observed statistically significant? Significant in any other sense?

DISCUSSION

12. Did you fulfill the specific purpose stated in your introduction? *E.g.* Do your results lend support to the specific purpose of your project (as stated in the INTRODUCTION)? Explain.

13. What additional experiments would you suggest to further the understanding of gravitropism in the hypocotyl, etc.?

14. Be sure to list references used and to cite references where appropriate in the body of the paper.

Lab Prep Materials

Required for growing the plants for both demonstrations and experiments

- Four inch square pots
- Jiffy mix or comparable seed starting mix (sphagnum, perlite, lime, fertilizer)
- Shallow plastic pans or flats to hold pots
- Distilled water source and distilled water in squirt bottles
- Bulk *Brassica oleracea* seeds (available on line as broccoli sprouting seeds)
- Vermiculite

Ancillary materials (per student group):

- 1 Petri plate, each filled with pieces of filter paper (to stick the cotyledons to the side of the film canister), a mini-protractor*, and a piece of clear graph paper* (the latter two are for making measurements),
- 1 250 ml or 500 ml squeeze bottle with dH₂O,
- 1 small scissors,
- 1 forceps,
- black electrical tape,
- 2 upright film canisters (1 already prepared as a demo and 1 for use by participants),
- 2 "Crucifer cross" canisters (1 for the demo and 1 for use by participants),
- 1 small plastic tray (to contain the squeeze bottle, empty sample canisters, scissors, forceps, canisters, Petri plates with filter paper, *etc*) for use by participants (Figure 4)



Figure 4: Trays holding equipment for student stations (left) and the tools themselves (right). Note that the protractor was created by photocopying a purchased protractor onto an acetate sheet. Smaller protractors can be easily created by reducing the image size during reproduction.

Other materials that students might request for their experiments:

• A Simple Clinostat. This is used to rotate canisters to equalize gravitational pull on all sides of the hypocotyl. A simple version can be built from an old stereo turntable modified as indicated in Figure 5. You can set something up like this or use a ten gallon white bucket cut to make a "sleeve" about 4 inches deep. This sleeve sits on the cabinet base, outside the turntable. The sleeve can be anchored using duct tape, etc. Coated wire is used to form pie shaped wedges inside the sleeve which should sit about 3/4 inch above the turntable when the sleeve is in position. A one quarter inch rubber mat is cut to the size of the turntable and glued to the turntable to provide sufficient friction to get the canisters to roll. Another way of doing this is to use a hot dog roller modified by disconnecting the heat source.



Figure 5: This stereo turntable has been fitted with stationary chambers to hold film canisters. This sits slightly above the turntable. Rubber matting glued to the turntable forces the canisters to slowly spin within their chamber.

• **Plant hormones** are available through biological supply houses and through chemical supply companies.

The hormones we use most often are auxin (IAA) and gibberillin. We provide students with concentrated solutions of the hormones (5 mM) which they must dilute to use. Hormones can be mixed with lanolin (w/w) to desired concentration. By mixing the known concentration with plain lanolin students can reduce the hormone concentration to the level(s) they choose. Hormone solutions are made up in distilled water and must be kept in the cold. Test the hormones for solubility in water before making the concentrated solution. If the hormone does not readily dissolve in water (try dissolving a few grains in water), try dissolving it in about 5 ml of 95% alcohol and then adding distilled water to make the appropriate concentration.

The hormone/lanolin mixture can be applied to one side of the hypocotyl, etc. using a needle or toothpick.

• Theater gel samples (Figure 6). These have specific wavelength transmittance and are often available at video recording supply companies, *e.g.* Full Compass in Madison, Wis. The sample gel colors indicated on another page in this chapter were selected because the key colors (blue, red and green) all had similar levels of brightness or light intensity but had varying ranges of wavelengths which were transmitted. Students can cap the film canisters with the gels and shine light from the side, above or below. Some students have cut slits or holes in the canisters, covered these with theater gels and directed light from various angles into the canister. Caution the students that the systems need to be TIGHTLY CAPPED AND/OR rubber banded if using theater gels. If not tightly sealed, the hypocotyls dry out and the experiment needs to be redone.



Figure 6: Transmission spectrum from gel sheets

(<u>http://www.rosco-ca.com/products/filters/index.cfm?fuseaction=Roscolux#Color</u>); useful for students to control light that their plants are exposed to.

• Centripetal Force Generator. Effects of centripetal force on the hypocotyl response can be measured using a modification of a table top centrifuge. This involves adding a 1 to 2 inch circular base of Styrofoam to the centrifuge top. If you cut the Styrofoam circle to the size of the top, you can attach it to the edge of the top with duct tape. Cylinders should be cut out of the Styrofoam to fit the canisters.

Differences in speed and centripetal force can be generated by attaching different sized cardboard circles to the styrofoam base (using double sided foam mounting tape). If you use a large cardboard circle (*e.g.* 3 feet in diameter or more) canisters can be placed at different radii from the center. A hot glue gun can be used to mount canisters to the cardboard circle.

- Amyloplast-"free" seedlings. Some students may want to test the effect of amyloplast movement on the response of the hypocotyl to gravity, *etc*. Some students managed this by growing *Brassica oleracea* seedlings in the dark and comparing the responses of the dark grown plants to those of others grown in the light. A few precautions, however:
 - Seedlings will etiolate and become very elongated and fragile if left for 5 days or more in the dark. Try to use 4 day light grown seedlings and 3 to 4 day dark grown seedlings.
 - The cotyledons will not expand until the dark-grown seedlings are exposed to the light. If the cotyledons are not expanded, they cannot be used to adhere to the filter paper. Modifications of experimental design must be made to account for this. As a result, if the students want to set up the experiment with the cotyledons adhering to filter paper, they will need to expose the etiolated plants to dim light until the cotyledons expand enough to use.
 - Students can do a qualitative measure of starch availability in cells by staining squash or free hand sliced preps of the hypocotyl with iodine and comparing the number of starch granules per field of view for etiolated vs. non-etiolated plants.
- **Tygon tubing, tacky wax, agar or dextran gel.** We've tried a number of mechanisms for suspending hypocotyls without cotyledons or suspending hypocotyls with cotyledons not adhering to the filter paper, *e.g.* It is possible to attach small pieces of tygon tubing to the sides of the canister using poster putty or tacky wax. To support the seedlings and to provide them with a water supply, these must be filled with a stable water source. Some students have attained this by inserting the hypocotyls through beads with openings approximately the same diameter as the hypocotyl and external diameters the same size as the tubing, essentially making a cork out of the bead/seedling. Others have used small amounts of tacky wax around the hypocotyl to cork the tubing. We have also packed the tubes with agar or hydrated dextran gel to support the seedling (see Figure 7).



Figure 7: Suspension of the seedling from its base rather than the cotyledons. The tube at right is attached to the wall of the canister.

Notes for the Instructor

Growing plants for gravitropism lab and demos

Start 7 days before plants are to be used.

USE DISTILLED WATER ONLY FOR WETTING SOIL AND WATERING!

- 1. Use 4 inch pots. Grow one pot per lab bench for student observations and 2 to 3 pots per lab for setting up the demo experimental canisters. You will need
- 2. Fill each pot loosely with Jiffy mix.
- 3. Place pots into flats or shallow plastic pans (see example on next page).
- 4. Fill flats with distilled water to cover about 1 inch of the bottom of the pots. (Note: if Jiffy mix is very dry, pots may begin to float. To avoid this put a weight, e.g. piece of acrylic sheeting or book, etc. on top of the pots.)
- 5. Allow the pots to soak up water overnight. (Then, remove whatever weight you may have used.) To be safe start some plants 6 days, some 5 days, and some 4 days before they need to be used. The plants will usually be ready for use about day 5 after planting. However, this can vary considerably based on the temperature in the growth chamber/area.
- 6. On each of the three planting days -- Sprinkle about 30 to 40 seeds on top of the Jiffy mix in each of eight of the pots.
- 7. Cover the seeds with a half centimeter thick layer of vermiculite and use a squeeze bottle of distilled water to thoroughly wet the vermiculite.
- 8. Keep the distilled water in the flat/plastic pan at about 1 inch deep during the growth period.
- 9. Place flats under grow lights (see pictures of our "state-of-the art" growth conditions on the next page) and grow for 4 to 6 days.

Week 1:

1) Introduce Brassica oleraceae:

To begin the lab, you should introduce the class to *Brassica oleraceae*. For example, remind students what the different parts of the plant are (in this 5 to 7 day old plant)

The hypocotyl is neither a stem nor a root, but can be thought of as the transition region between these in the developing plant. It can therefore respond differently than either stem or root to various

stimuli. You can think of the esophagus, stomach and small intestine as an analogous system. All of these are connected to each other, yet each is distinct in its structure, function and responses to stimuli. The same is true of root, hypocotyl, stem and cotyledon.

Using overheads of text book cross sections or drawing diagrams of cross sections through both dicot root and stem helps students understand that the hypocotyl is a zone of transition of internal structure especially of the xylem and phloem organization.

- a) Have students turn to the second page of the lab exercise. When working with a new or unknown system or organism, scientists usually begin by gathering baseline data and testing their own biases by making objective observations of the behavior, etc. of the system or organism under study. The exercise on the second page is designed to provide you with some baseline data and to allow you to begin developing hypotheses and the rationale for these. It should also help you determine if you have any biases or "preconceived" notions of how the system or organism "should act".
- b) Show students how the demonstration (and experimental) canisters were set up. Strips of filter paper are cut to size (approx. 3/8" x 1 and 3/4"). Strips are placed in a Petri dish with distilled water. A single strip of wet filter paper is oriented vertically along one side of the film canister. Approximately 2 ml of distilled water is added to the bottom of the film canister (enough to make a dime sized drop). A *Brassica* seedling is cut and its cotyledons are placed against the wet filter paper near the middle of the strip. Surface tension holds the filter paper to the side of the canister and the cotyledons to the filter paper.

Press down gently on the cotyledons with a dull forceps point to increase surface tension contact between water in filter paper and the cotyledons. Cap the canister tightly. It takes from 3 to 6 hours to get significant bending in seedlings oriented in this direction. In setting up demos for the lab, it is possible to set them up the night before. Given sufficient water, seedlings can survive in canisters up to 3 days. By about the 3rd day, they will start to regenerate roots.

c) IN GROUPS OF 3-4 have each group first decide what they think the response of the hypocotyl will be in Experiment 1 and what their rationale is for this decision.

Once they've come to some agreement, they should draw their predicted results on the blackboard.

To help keep data more organized and on the same scale, draw the following on the board and ask them to draw the hypocotyl as they expect to see it when they open the canister.



- d) Once all drawings are up on the board, ask each group its RATIONALE (reasoning) for the HYPOTHESIS (drawing) that they proposed. In other words, why (for what reasons) do they think it will respond the way they have it drawn. What might cause it to do this? What information do they have that the proposed mechanism might be operational in the hypocotyl response?
- e) ONLY after all groups have explained rationales pass out the canisters and let them open them.
- f) Now have them all return to the board and draw (using a different color chalk) what they see when they look in the canister. Tell them to try to represent the angle and direction of growth of the hypocotyl as accurately as possible!

2) The Crucifer Cross Experiment (Experiment 2).

Tell your students how the crucifer cross demonstration canisters were set up.

Attach a film canister top to one side of a canister using black electrical tape (available at hardware and many other stores). This will allow you to orient the canister on its side. To set up the crucifer cross (crucifer because *Brassica* is in the group Cruciferae or the mustard family) set the canister upright and orient 3-4 wet filter paper strips along the canister walls. If the circular opening were viewed as the earth or globe, the filter paper strips should be placed at the north and south poles and at the east and west extremes of the equator. Again, apply a seedling near the middle of each filter paper strip so that the cotyledons stick to the wet filter paper by adhesion/cohesion. Add about 1-2 ml of water to the bottom of the canister (about a dime sized drop). Cap the canister and set it on its south side, i.e. resting on the cap that has been "glued" to one side. (Again, it takes 3 to 6 hours for a good response. It is possible to set these demos up the night before.)

Draw the following on the board and have students again work in groups to determine how the hypocotyls will respond.



- a) Students fill in their hypothesis or prediction first in one chalk color.
- b) When all groups have their predictions on the board, the students explain their rationales for proposed hypocotyl responses.
- c) Pass out the new canisters and have students open them and draw what they actually see in a different chalk color.
- d) After all data are on the board, discuss the data and how to begin analyzing it, for example:

- Different kinds of growth can occur in organisms, i.e. growth by addition of numbers of cells and growth by elongation or expansion of existing cells. (Given the location of the response, i.e. below the apical meristem or the zone of cell division, the hypocotyl response is the result of cell elongation or expansion.)
- There is obviously variability among data and this will need to be controlled for in an experiment (*e.g.* increase numbers of samples tested, use all the same size, age, etc.).
- The method for measuring change from the original position of hypocotyl will be important especially when using differential concentrations and you want to compare the different treatments to the control. How to measure is the most difficult aspect of this experiment and one that students often do not address well. In response *vs* no response type experiments, changes from the vertical in hypocotyls set up as in the south position of the crucifer cross would be easy to identify. If on the other hand you want to measure amount of change from one treatment type to another, i.e. variability in response, students will need to come up with mechanisms to measure that variability.
- Data may be missing , e.g. hypocotyls may fall off filter paper, etc. Increasing the number of replicates will help solve his and some of the other problems noted above. (You can also remind students that they can put more than one hypocotyl in a canister and this will reduce numbers of canisters they will need. It may also reduce variability resulting from possible environmental differences among canisters.

3) Students should then review their prelab exercises and use this information to come to a consensus in their groups on: .

a) a hypothesis or question for an experiment

- b) an experimental design to test the hypothesis/question
- c) appropriate controls
- d) a reasonable method for measuring change/growth.
- One way of forcing this last issue is to give them about 15 min. in each group to develop a method to measure the response. Then have the class come together in large group to develop a consensus strategy (or two) that all will use. Remember there are a number of different factors that can be measured, e.g. angle of bending, amount of elongation, etc.

In developing controls, it is important to keep in mind that following differentiation, cells have a maximum upper limit on the amount of elongation/expansion that can occur. It is therefore necessary to control for age of plant and length of hypocotyl in setting up experiments which require comparing differences in angle and/or extent of elongation. If time is constrained, do this at the beginning of week 2.

Remind students to keep their hypotheses simple and testable.

4) The sample hypothesis exercise -- week one.

Have students brainstorm what should be contained in each section of a proposal. Then have them review a sample proposal. We use a proposal that is in the general format we'd hope to get week 1 in the lab.

Students work individually to review this. Then have them come together in their group and compare their reviews. Next the whole lab discusses what's good about this proposal and what should be changed, added, etc.

[While on the surface, the sample proposal looks pretty good, there are a number of problems with it. In particular, the design while good, can in no way tell the students if "auxin causes the gravitropic response in broccoli". It can, however, tell them if artificial application of auxin can alter the gravitropic response. Your review suggestion in this case would be to keep the design and change the hypothesis and rationale to a purpose statement. E.g. The purpose of this experiment is to determine what effect(s) artificial application of auxin to the hypocotyl will have on the observed gravitropic response. The rationale would be similar but slightly different, i.e. auxin has been shown to affect gravitropic response in stems. Because the hypocotyl is a transitional region between stem and root, it may also be similarly affected by auxin.

BE CAREFUL, when you indicate to students that their design does not fit their hypothesis, their first inclination is to throw out the design. You need to make it clear, that more often than not, the design is good and they need to restate their purpose or hypothesis more narrowly.]

5) Have student groups begin working their own proposals.

Note: Students will want to know what equipment is available to them before beginning proposals. Let them know that they shouldn't feel limited by this. If they have an idea and want to know if we have the equipment to do this have them ask you. *You should do this because if you demonstrate what's available first, the students become more limited in their ideas.* Ask them to check with you during hypothesis development if they are concerned about possible equipment, etc.

Indicate that you can let them know if the equipment their proposing (or something similar) will be available. As needed, on a group by group basis, you can then demonstrate or describe different kinds of materials which could be made available, e.g. lanolin with hormone. structural support mechanisms, turntables, theater gels, light sources, etc.

The proposal:

Students should develop their hypothesis and experimental design with list of "equipment and supplies needed" attached and hand these in at the end of the lab period.

Students must hand in proposals before they leave.

Week 2:

Before Lab - Review the proposals.

- a) Concentrate on whether the design matches the hypothesis. Generally, students develop good designs for experiments, they just don't happen to address the hypothesis they propose. Your review suggestion in this case would be to keep the design and change the hypothesis and rationale to fit the design. BE CAREFUL, when you indicate to students that their design does not fit their hypothesis, their first inclination is to throw out the design. You need to make it clear that the design is reasonable but that they need to restate their purpose or hypothesis more narrowly.
- b) Again, one big question to overcome is "how to measure response?" You may need to have this discussion again in week 2. Measurement becomes easier if you're looking for a response vs no response based on your design. One possible response vs no response system (devised by past students) involves using hypocotyls oriented upside down to gravity (like the bottom ones in the crucifer cross example). These you would expect to remain straight over time. Application of substances to the sides, etc. which changed this orientation would be easier to distinguish.
- Measuring becomes much harder in this system if your design asks you to look for degree of response or difference in response between two treatment types. This latter question requires that the amount of response or bending be measured. One possible mechanism for determining amount of response is approximating total angles of curvature. Remember, growth in these systems is by extension or elongation of individual cells already present in the hypocotyl, not by addition of cells. How much each individual cell elongates is under control of internal cell signals, as well as, how much water was available to the given plant.

At the beginning of the lab

- 1) Give students a general idea of common review comments (e.g. alter purpose/hypothesis rather than design).
- 2) Then show them what types of equipment are available for the particular project designs they have indicated.

Have fun!

APPENDIX B

Stocks and Solutions

EDTA

EDTA is a general cation chelator. It chelates positively charged ions. To chelate means to take the ions out of solution and make them unavailable for other reactions. Stock solution is 50 mM EDTA.

Soak plants or plant parts in 5.0mM EDTA for between 2 and 10 minutes.

EGTA

EGTA is a Ca⁺⁺ ion chelator. It chelates Ca⁺⁺ ions specifically. To chelate means to take the ions out of solution and make them unavailable for other reactions. Stock solution is 50 mM EGTA. Soak plants or plant parts in 5.0mM EGTA for between 2 and 10 minutes.

Calcium Chloride (CaCl₂)

Use to supplement Ca⁺⁺ in plants or plant parts. Stock Solution is 50 mM. Use at concentrations of between 0.5 and 5 mM. Soak plant parts for between 2 and 10 minutes.

Plant Hormones

Auxin (Indole Acetic Acid or IAA)Dilute to 3 mM-0.2 mM range for useDilute by mixing with lanolin.Concentrations provided in lanolin:5000 ppm = 2.8 mM500 ppm = 0.28 mM

Gibberellin Dilute to 0.1 - 0.005 mM range for use. Dilute by mixing with lanolin. Concentration provided in lanolin: 500 ppm = 0.14 mM.

Apply hormones in very small amounts using a fine pointed dissecting needle or toothpick.

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Jean Heitz is a Distinguished Faculty Associate in Zoology at the University of Wisconsin-Madison and has worked with a two-semester Botany/Zoology introductory sequence for majors since 1978. Her key roles have been in development of active learning activities for discussion/recitation sections and open-ended investigations for laboratory sections. For over 10 years, she taught a graduate course in "Teaching College Biology". She is also the lead author of the Practicing Biology student workbooks that accompany Campbell et al.'s Biology 2008 and S. Freeman's Biological Science, 2008. Her current interests are in continuing to improve on these efforts.

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