Topic 09: Plant Tropisms & Hormones

A. Introduction

By conventional thinking, plants are not as exciting as animals due to their apparent lack of behavior. However, plants exhibit a range of behaviors that, considering the physical restraints of being rooted in place, are rather remarkable.

Most plant movements generally fall into two classes: (1) movements based on growth (e.g., TROPISMS) and (2) movements based on changes in turgor. Examples of the latter include the closing and opening of venus flytrap leaves (*Dionaea muscipula*), the curling of sundew leaves around insect prey (species of the genus *Drosera*), the folding of leaves of the "sensitive plant" (*Mimosa pudica*) in response to stimuli such as touch (Fig. 1), and the apparent solar tracking movements of young sunflower plants (*Helianthus anuus*) or the petioles of leaves of many species to optimize the blades' interception of sunlight. These bending movements result from changes in cellular water content (i.e., changes in turgor) of tissues on one side or region of the particular organ (with bending occurring *towards* the side of cellular water loss).

Fig. 1: *Mimosa pudica* (sensitive plant) plant before and after experiencing a touch. The closed leaves are the result of changes in water content in the leaves.

The tropisms differ from turgor movements in that tropisms are irreversible changes in the orientation of the particular organ, due to unequal growth (not changes in turgor) on one side of the organ exposed to an environmental stimulus. **PHOTOTROPISM** and **GRAVITROPISM** are directional growths ("movements") in response to light and gravity, respectively. Phototropism is responsible for orienting the shoot tissues of the plant towards the window, out from beneath the shade of a fallen tree in the forest, or up if the shoot had fallen over to a horizontal position due to some disturbance. Gravitropism is best known with roots (which exhibit *positive gravitropism*), but shoots also exhibit a form called *negative gravitropism*. That is, roots grow towards gravity and shoots grow away from gravity. In roots, gravity is perceived in the root cap and removal of the root cap eliminates this gravitropic response. Current understanding of gravity perception suggests that the settling of amyloplasts in special root cap cells triggers the physiological recognition of gravity and stimulates growth in the direction of the settling organelles. A third tropism is **THIGMOTROPISM**, which is movement towards or away from a solid object that the plant has touched. Some examples include the tendrils of pea

plants or grape vines wrapping around a support. These tropisms, like all growth phenomena in plants, are regulated by hormones.

B. Phytohormones

This lab is also about plant hormones, which regulate not only the tropisms, but all growth phenomena in plants. Currently six hormone classes have been identified in plants: Auxin, Cytokinin, Ethylene, Gibberellin, Abscisic Acid, and Brassinosteroids. All of these chemicals have specific functions critical to the completion of the plant life cycle. Auxin, cytokinin and gibberellin all contribute to the growth of the plant. Auxin has been identified as a major component of differential growth associated with the tropic movements. Gibberellin is known to stimulate germination but also stimulates stem elongation.

C. Hormone Regulation of Growth – Gibberellins: (Work as a table, or group of 8)

Peas (*Pisum sativum* of the legume family) with genetic differences (cultivated varieites *P. sativum* cv. 'Alaska' = tall variety, and *P. sativum* cv. 'Little Marvel' = dwarf variety) will be used to investigate the role of Gibberellins in shoot growth.

1. Each table has four pots each of 'Little Marvel' and 'Alaska' variety pea plants.

2. Remove the smallest plant(s) from each pot, keeping six uniform plants.

3. Designate the following treatments (write on the labels) for both the Little Marvel and Alaska pots:

Control (water plus tween-20)
100ppm GA plus tween-20
1000ppm GA plus tween-20
1000ppm B-Nine plus tween-20

4. Identify and mark the first and second internodes with a marker. These are the youngest internodes closest to the growing tip.

5. As a table, record the following measurements for each plant:

- Length of the first (closer) internode extending from the apex
- Length of the second (further) internode from the apex

6. Determine the average lengths for each the first and second internodes in the following tables.

7. Obtain the data for both pea varieties with the other members of your table.

8. Take the GA and B-Nine treatment plants to the hall or outside and apply the foliar spray treatment.

9. Be sure to completely cover the leaves of all pea plants in the pots. Be careful not to cross-contaminate with drift from the other groups that may be spraying.

10. Return the pots to the greenhouse for light and watering (by staff) until the next lab period.

11. After 1 week, measure the lengths of the first and second internodes. Record in the appropriate tables. KEEP IN MIND THAT THESE INTERNODES WILL NO LONGER BE THE FIRST AND SECOND INTERNODES.

Formulate hypotheses about the effect that these treatments will have on stem growth. In the following tables, format your hypotheses as follows: no growth = "-", normal growth = "+", more growth = "++", even more growth = "+++".

Table 1. Hypotheses about growth in *Pisum sativum* cv. 'Alaska' observed after one week of the following treatments.

treatment	growth observed generally
Control (water)	
100ppm GA	
1000ppm GA	
1000ppm B-Nine	

Table 2. Hypotheses about growth in *Pisum sativum* cv. 'Little marvel' observed after one week of the following treatments.

treatment	growth observed generally
Control (water)	
100ppm GA	
1000ppm GA	
1000ppm B-Nine	

How do expect the percent elongation to compare between internodes 1 and 2? That is, in which internode do you expect to see the greatest change in length?

Justify your hypotheses:

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AFTER ONE WEEK

Do your results support or refute your hypotheses?

How did the plant growth change in response to gibberellin application?

Did both varieties respond in the same way to GA?

Does changing the concentration of the GA make a difference in the growth?

Is there a difference between the two internodes measured?

Explain why any differences in the internodes might have occurred.

How did the varieties respond to B-Nine application?

Formulate a hypothesis to explain your results?

Table C1. 'Little Marvel' variety results with control treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	**day 7**	**% change**	**day 0**	**day 7**	**% change**
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C2. 'Alaska' variety results with control treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	**day 7**	**% change**	**day 0**	**day 7**	**% change**
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C3. 'Little Marvel' variety results with GA 100 ppm treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C4. 'Alaska' variety results with GA 100 ppm treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C5. 'Little Marvel' variety results with GA 1000 ppm treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C6. 'Alaska' variety results with GA 1000 ppm treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C7. 'Little Marvel' variety results with 1000 ppm B-Nine treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

Table C8. 'Alaska' variety results with 1000 ppm B-Nine treatment.

	Internode 1 (distal) length			Internode 2 (proximal) length		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						

D. Phototropism in the Shoot: (Work in groups of 4)

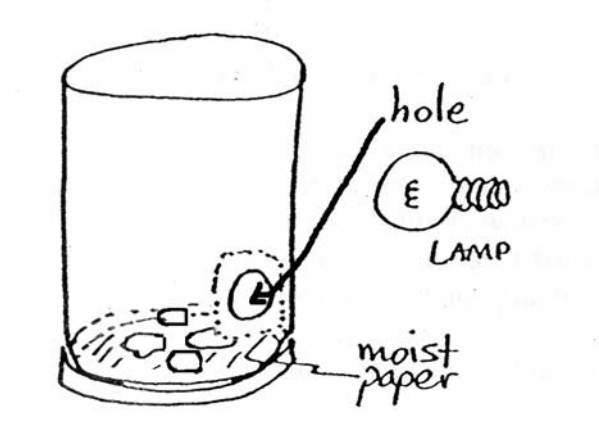
You will using radish (*Raphanus sativus* of the mustard family) seedlings for this experiment. We will collect data after the experiment has run for one week.

1. Set up 6 film canister "**LIGHT CHAMBERS**". Four should have one hole in the side, one should have a hole in the top and one should not have any hole present.

2. Remove the lid and cut several layers of paper towels to fill the interior area of the lid (approximately 10 paper towel layers in thickness). Add water to soak the paper towels.

3. Locate the following light filters: red, green, blue, and clear (represent white light). Place one filter in each of the canisters with the hole in the side so that the filter covers the hole. Secure with tape if required. Locate the colored tape on the bench, place a small piece on top of your canister to label it with your group name and the color of filter.

4. Add 4 radish seeds to the moist filter paper and then lock the barrel of the canister onto the lid. See the image below for reference. Be sure to not invert the canister from this point on!



5. Expose the canisters to a light source so that the hole is aimed toward the light source (as best as possible).

6. Repeat the procedure with the canister with a hole in the top and the canister without any holes. Cover the hole on top with saran wrap or a clear filter (secure with tape). Label the canisters appropriately.

7. Prepare a control setup: use a glass beaker, place paper towels in the bottom, soak them, add 4 radish seeds, and cover with saran wrap (secure with tape). Place under the light source.

8. Observe the results paying special attention to height, color, angle of growth, cotyledon development, and the apical hook. Record your results in the table below:

Table D. Growth and curvature of seedlings after 6-7 days.

Treatment	Height	Angle (relative to control)	Color	Cotyledon/hook Development
Control				
Side/white				
Side/green				
Side/blue				
Side/red				
Top/white				
Dark				

Explain how each treatment affected the directional growth of the shoot:

Top/white

Side/White

Side/Green

Side/Red

Side/Blue

Dark

What characteristics are associated with photomorphogenesis?

What characteristics are associated with skotomorphogenesis?

E. Gravitropism vs. Phototropism:

1. Radish (*Raphanus sativus* of the mustard family) seeds have been previously planted in 14ml tubes containing vermiculite and grown under florescent lights. Select 2 of the tubes and label them appropriately for identification by your group.

2. Select one plant/tube combo to place horizontally in a dark environment. This is your gravitropism response (control) sample.

3. Place the second plant/tube combo horizontally in the prepared box that has a light source beneath it. This is your phototropism vs. gravitropism competition box.

4. Once all samples have been added, the boxes should be sealed for 4 to 7 days (refer to your instructor for specific instructions.

Predict how the plant in the horizontal/dark environment will respond.

Predict how the plant in the horizontal/ unidirectional light environment will respond.

5. Observe the phenotype of the specimens. Notice any curvature, severity of curvature, or other developmental changes present.

How did the dark/horizontal plant respond to the treatment?

How did the light/horizontal plant respond to the treatment?

In each scenario, was the tropic response positive or negative?

Which tropism was dominant?

How do the tropisms assist the shoot (think development)?

How do the tropisms assist the root (think development)?

F. Hormone Regulation of Growth – Auxin (Work in groups of 4)

We will use common bean (*Phaseolus vulgaris* of the legume family) seedlings for this experiment.

1. Obtain three bean plants growing in flats with 5 rows each.

2. The middle row is a control (where no auxin will be applied).
   Label the tag "**control, no treatment**".

3. The two rows on either side are for each of the two groups of 4 students per lab bench. Each group of 4 shares the control row, and will work separately with the two additional rows on their side of the flat for the following treatments:

   3a. Label the tag of the next row "**Auxin, One Side of Shoot**"
   Apply auxin-lanolin paste with a toothpick or similar instrument to only one side (the side closest to the tag) of the stem for 5-10 mm max just below the shoot apex of each plant in that row.

   Mark the stem just above the auxin application on both sides of the stem.

   3b. Label the tag of the last row "**Auxin, Complete Ring Around Shoot**".

   Apply auxin-lanolin paste with a toothpick or similar instrument in a ring around the stem for 5-10 mm max just below the shoot apex of each plant in that row.

   Mark the stem just above the auxin application on both sides of the stem.

4. Allow the plants to grow until next lab and observe the results.

Table F. Hypotheses about growth in *Phaseolus vulgaris* seedlings in response to differential applications of auxin-lanolin paste after one week.

treatment	Any curvature of stem detected?	Direction of any curvature?
Control (no application)		
Auxin, on one side of shoot		
Auxin, complete ring around shoot		

After 6-7 days, hHow did the application of auxin change the growth of the plant? Draw any interesting results.

Is there a difference in development between treatments 3a and 3b? Explain?