Topic 11: 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. For example, sunflowers exhibit a remarkable, and repetitive movement called "sun-tracking" or *heliotropism* during which the shoot apex containing the floral inflorescence orients itself to the East before sunrise in order to receive the first light of dawn and throughout the day the flower moves from East to West following the sun. This movement keeps the flower perpendicular to the sunlight. Obviously plants are not as immobile and unresponsive to the environment as it first appears.

Some plants have rather dramatic behaviors in response to touch. These types of movements are known as **THIGMOTROPIC** movements. Some examples include the movements of the Venus Flytrap leaves, pea leaf tendrils wrapping around a support, and the folding of leaves seen in *Mimosa pudica* (the sensitive plant). These movements occur in response to touch and may be the result of changes in cellular water content or cellular growth.

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

The most basic and widespread of all plant tropisms are **PHOTOTROPISM** and **GRAVITROPISM**. These 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. That is, roots grow towards gravity. 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 organelles in special root cap cells triggers the physiological recognition of gravity and stimulates growth in the direction of the settling organelles.

Changes in growth are the result of plant hormones that are synthesized and distributed in response to environmental and internal signals. 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.

In this lab, you will be investigating the effect of light, gravity, and specific hormones on plant development. Fast Plants (*Brassica rapa*) which have a very rapid lifecycle and respond quickly to environmental cues will be used for the tropism investigations. Peas with genetic differences (cultivated varieties 'Alaska' = tall, and 'Little Marvel' = dwarf) will be used to investigate the role of Gibberellins in shoot growth. Optionally, a strongly apical-dominant species, tobacco, will be used to investigate the role of auxin in apical dominance.

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

1. Set up 5 "**LIGHT CHAMBERS**" by using a film canister that has a single hole punched in the side.

2. Set up 5 light treatments by placing the appropriate cover/cellophane filter over the hole of each as follows: a) DARK (foil), b) white (clear cellophane over the hole), and c) Red, d) Blue, e) Green.

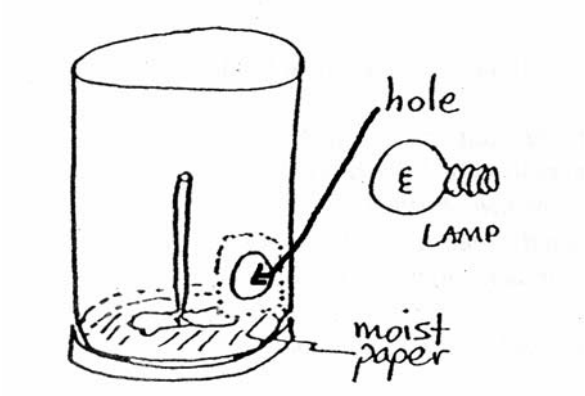
3. Fit a layer of moist paper to the inside of the lids.

4. Prepare **one Fast Plant (*Brassica rapa*) for each chamber** by de-rooting it and placing the cotyledons on the moist filter paper. Complete quickly to avoid desiccation.

5. On the cap, mark the side of the hole (light source) so that we can know if the hypocotyl grew towards or away from the light source.

6. Measure and record the angle between the hypocotyl and the moist filter paper. (It should be approximately 90°.)

7. Place the canister over the plant (inverted, see figure below) and put in front of the light source for 90 min. THE PLANT CANISTER SHOULD BE 20cm FROM THE LIGHT SOURCE, AND A WATER HEAT SHIELD HALF-WAY BETWEEN THE LIGHT AND THE CANISTERS.



8. After 90 min, measure the curvature towards or away from the light, if any. Make observations and drawings of the plant.

9. Determine the rate of curvature as "degrees/minute." Calculate the average rate of curvature for all three plants of the same treatment. Record your data in the table below.

10. Pool the treatment data with the other groups.

Which way and where did the hypocotyl curve?

Can any wavelength stimulate phototropic movements?

What wavelengths were most effective?

Which wavelength was least effective?

What color pigments might be involved?

How do phototropic movements benefit the plant?

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Table 1. (Give this a caption yourself)

Treatment	Initial Angle	Final Angle	Difference (degrees," –" or "+")	Time (min)	Rate (deg/min)
White					
Dark					
Red					
Yellow					
Green					
Blue					

C. Gravitropism in a shoot?

1. Set up 1 "GRAVITY CHAMBER" as below. The set up is as above for the light chambers, only that the film canister has no hole in it. No need for the cotton in the bottom as drawn in the figure.

2. Mark the lid on the side that will be DOWN.

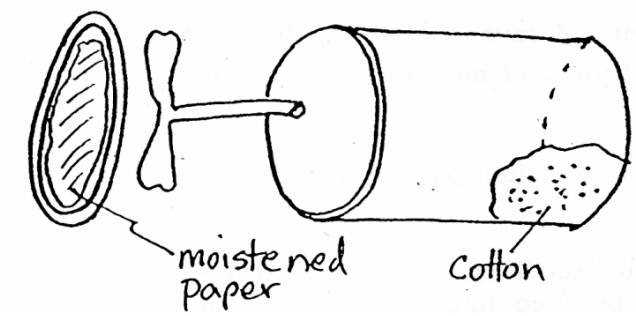
3. Rest the chamber horizontally, onto choc-blocks to keep the 'DOWNSIDE DOWN.'

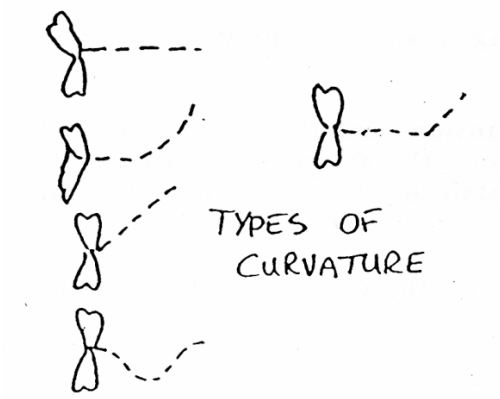
4. Time how long it takes to reach 45-90 degrees curvature, if any. Check every 20 minutes. Calculate the rate of curvature if any.

5. If there is curvature, indicate in which direction (away or toward gravity) and make drawings to indicate WHERE in the hypocotyl the curvature was.

6. **MAKE A WET MOUNT** using water at first. ATTEMPT TO DETERMINE if the curvature was achieved via cell production and/or cell expansion. Add toluidine blue secondarily if necessary.

Determine average cell size (length) and number through the region of curvature. Make drawings.





Which way and where did the hypocotyl curve?

If curvature, would you call this negative or positive gravitropism?

If curvature, how might gravitropism in the shoot help a plant?

How does gravitropism in a root help a plant?

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

1. Each table has four pots of Little Marvel and four pots of Alaska 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)
100 ppm GA plus tween-20
1000 ppm GA plus tween-20
1000 ppm 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. Do this for all plants (treatments) of both pea varieties.

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. KEEP IN MIND THAT THESE INTERNODES WILL NO LONGER BE THE FIRST AND SECOND INTERNODES.

Table D1. '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						

Table D2. '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						

Table D3. '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						

Table D4. '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						

Table D5. '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						

Table D6. '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						

Table D7. 'Little Marvel' variety results with 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						

Table D8. 'Alaska' variety results with 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						

What were your starting hypotheses regarding the effect of each treatment on each variety?

Did your results

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?

E. Gravitropism of the Root: (Work in groups of 4)

1. Each group will be given one Petri dish with three Fast Plants growing on it.

2. Observe the growth of the roots and shoot with respect to how the plates were oriented. In the absence of impeding structures (soil) is there an obvious orientation of the roots? Do any of the roots bend? If so suggest a hypothesis for it.

3. Using a ruler and a black marker (fine point) draw a line that follows the line of the root. The line should be directly on top of the root and parallel to the direction of growth. Focus on the apical root tip region. This will be you base line.

4. Use a finger clamp to mount the Petri dish/Fast Plants so that the line drawn over the primary root growth is horizontal (parallel with the ground). Be sure to clamp the plate snuggly, but do not over tighten or you will break the plate.

5. Observe the orientation of the root growth every 30 minutes for 120 minutes and draw a new line through the newly oriented section of the root for each time point.

6. Which region of the root is changing direction, the tip or the region near the base? Why? Which direction does the root reorient to (up or down). Can you think of a way to measure root growth in the absence of gravity?

7. Using your protractor, measure the angle between the original base line and the new line at each 20-minute mark. Calculate the rate of curvature as Degrees/minute.

8. Pool your treatment data on the board with the rest of the class. What trends do you see?

Time (min)	Plant 1	Plant 2	Plant 3	Plant 4
0				
30				
60				
90				
120				
Rate (°/min)				

How does gravity influence root growth?

How does this response benefit the plant?

What type of response to gravity would you expect in a shoot?

F. The Role of Auxin in Stem Growth

1. Obtain two pots of sunflowers per group of four students.

2. Identify the shoot apex region of the sunflower.

3. Apply a lanolin paste containing 1000ppm auxin (IAA) to one side of the stem of each sunflower plant in the pot. Locate this application approximately 1 cm below the shoot apex. Be sure to keep the lanolin paste to one side of the stem.

4. Mark the stem with a sharpie (black marker) on the opposite side of lanolin application.

5. Apply lanolin paste to one side of the stem of the sunflower plants in the second pot approximately 1 inch above the soil line. Mark the stem at the site of application. Be sure that the lanolin is on one side of the stem only.

Hypothesize what you expect to happen to the growth pattern of the plants.

7. Next Week: observe the plants for any changes in growth.

What happened at the region below the apex?

What happened at the region above the soil line?

How do the results of the two applications compare?

How do the treated plants compare to control plants?

What is auxin's role in stem growth?