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 head moves from East to West following the sun. This movement keeps the head 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** or **THIGMNONASTIC** (or TURGOR) movements. Some examples include the movements of the Venus Flytrap leaves (thigmonastic), pea leaf tendrils wrapping around a support (thigmotropic), and the folding of leaves seen in *Mimosa pudica* (the sensitive plant) (thigmonastic). These movements occur in response to touch and may be the result of changes in cellular water content or cellular growth.

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

The most basic and widespread of all plant tropisms are **PHOTOTROPISM** and **GRAVITROPISM**. These include directed growth ("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 include: 'Alaska' = tall, and 'Little Marvel' = dwarf) will be used to investigate the role of Gibberellins in shoot growth. Optionally, a strongly apical-dominant species 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. One chamber will not have a hole in it…use this one for DARK.

2. Set up 5 light treatments by placing the appropriate cover/cellophane filter over the hole of each as follows: a) DARK (no hole in side), b) white (clear cellophane over the hole), and c) Red, d) Blue, e) Green.

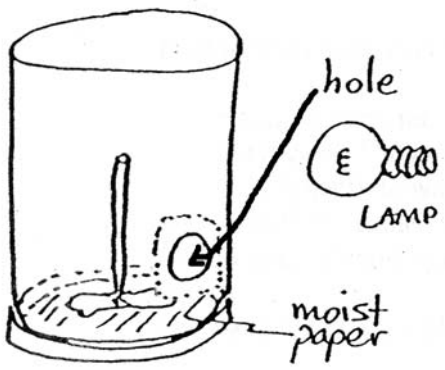
3. Fit two layers of moist filter paper or paper towel to the inside of the lids.

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. Make sure the hypocotyl is perpendicular to the canister cap.

5. On the cap, mark the side of the hole (light source) with white-out so that you can determine if the hypocotyl grew toward 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 15cm FROM THE LIGHT SOURCE, AND A WATER HEAT SHIELD BETWEEN THE LIGHT AND THE CANISTERS.



8. After 90 min, measure the curvature toward 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. If time allows, pool the treatment data with the other groups on the board.

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 pigments might be involved?

How do phototropic movements benefit the plant?

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Table 1. (Give this a TITLE AND CAPTION yourself)

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

C) Hormone Regulation of Growth – Gibberellins: (Work as a table)

1. Each table has four pots each of 'Little Marvel' and 'Alaska' variety pea plants and will be responsible for the measurement of EIGHT plants in each pot.

2. If there are more plants than EIGHT per pot, remove the extras without disturbing the remaining 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. SUGGESTION: use the marker to mark the leaves at the base of the second internode! LABEL the leaves with the number of the plant.

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

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						

Part C: Thought Questions….

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

Did your results support your hypotheses? Explain:

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?

D) Gravitropism vs. Phototropism:

1. Radish 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 1 to 3 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)?

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

1. Obtain three sunflower plants growing in individual pots.

2. Label them as:

#1 = Control, Lanolin application to one side of the hypototyl

#2 = + Auxin, One Side of Hypocotyl

#3 = + Auxin, Complete Ring Around Hypocotyl

3. Add auxin as described, one inch below the apex. Apply with a toothpick. For tube #2, preferentially add the auxin ONE INCH below the apex. Repeat with application one inch below the apex for treatment #3.

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

How did the application of auxin change the growth of the plant?

Is there a difference in development between tubes #2 and #3? Explain.