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.

Some plants have rather dramatic behaviors in response to touch. These types of movements are known as **THIGMOTROPIC** or **THIGMONASTIC** movements. Thigmotropic movements are irreversible differential cellular growth in response to touch, whereas thigmonastic movements are reversible turgor-driven movements in response to touch. 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).

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 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 is perhaps partially responsible for a shoot that has fallen over to a horizontal position due to some disturbance to right itself and resume growing up.

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 amyloplasts 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. Radishes (*Brassica rapa*) seedlings which have a very rapid lifecycle and respond quickly to environmental cues will be used for the tropism investigations. Peas with genetic differences (cultivated varieites 'Alaska' = tall, and 'Little Marvel' = dwarf) will be used to investigate the role of Gibberellins in shoot growth.

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

1. Perform this setup at the beginning of the lab period. 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 (no hole in side), 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 radish seedling (*Brassica rapa*) for each chamber** by de-rooting it and placing the cotyledons on the moist filter paper. Complete quickly to avoid desiccation.

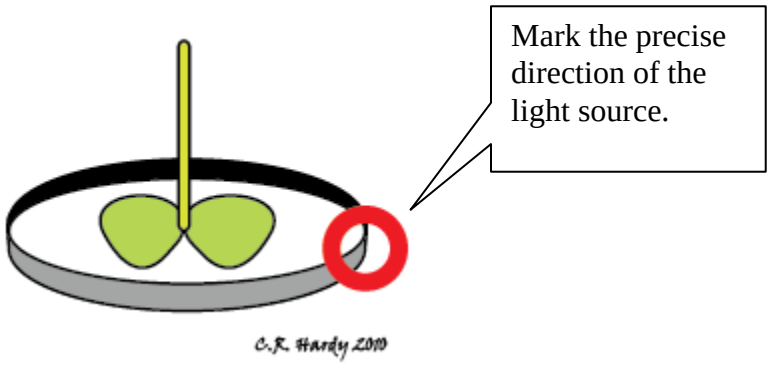


Figure 2. De-rooted radish seedling adhering to wet paper on inside of upside-down film canister lid.

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. In Table 1 (top row), make a drawing of the upside-down seedling and its starting orientation as if the light source is to the right side of your paper. Pay careful attention of the angle of the hypocotyls with respect to the table and the direction in which the light lays (It should be approximately 90° - if it's not, then that's okay, but you will rely on your drawing to see how much it subsequently curves (if any) towards or away from the light).

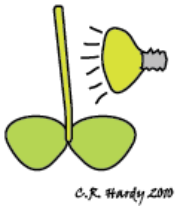
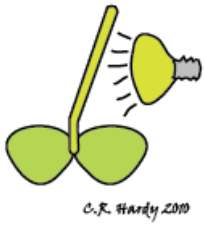
 Sample Initial	Dark Initial	White Initial	Red Initial	Blue Initial	Green Initial
 Sample Final	Dark Final	White Final	Red Initial	Blue Final	Green Initial

Figure 2. Appearance and curvature of radish hypocotyls before and after exposure to various light regimes. Light source presumed to be to the right in figure.

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 10cm FROM THE LIGHT SOURCE WITH THE WATER HEAT SHIELD BETWEEN THE LIGHT AND THE CANISTERS.

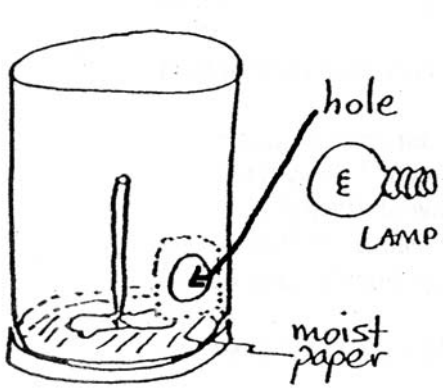


Figure 3. Complete set up.

8. After exactly 90 min, redraw your seedling in the bottom row of Table 1 with respect to the light source and determine how much curvature there was.

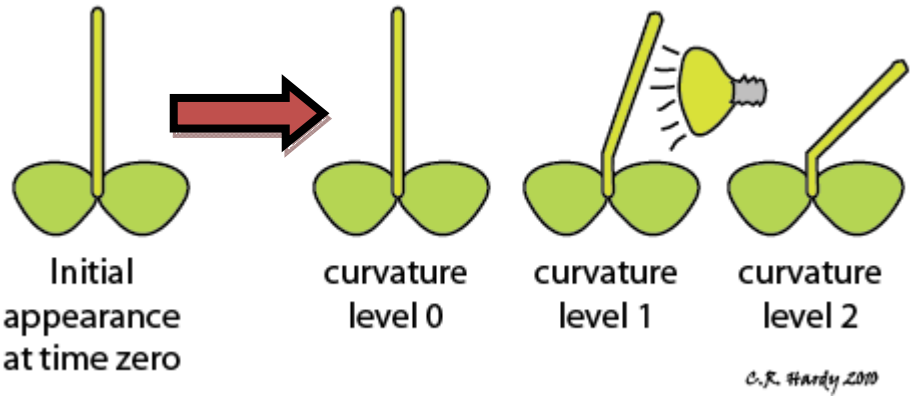


Figure 4. How to quantify the level of curvature. If curvature was towards the light, source, then these numbers (0, 1, or 2) are positive. If away from light, then numbers are negative.

Table 1. Degree and direction of curvature to or from a light source of radish hypocotyls after 90 minutes subjected to that light source. Key as follows: -2, strong curvature away from opening (usually the light source except in the dark treatment), -1 = weak curvature away, 0 = no curvature, 1 = weak curvature towards light source, 2 = strong curvature towards light source.

	Group1	Group 2	Group 3	Group 4	Group 5	Group 6	Class Sum Total
Dark							
White Light							
Red Light							
Blue Light							
Green Light							

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. Sum the totals for each treatment from all groups to quantify which light treatment caused the greatest and least amount of phototropic response.

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?

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

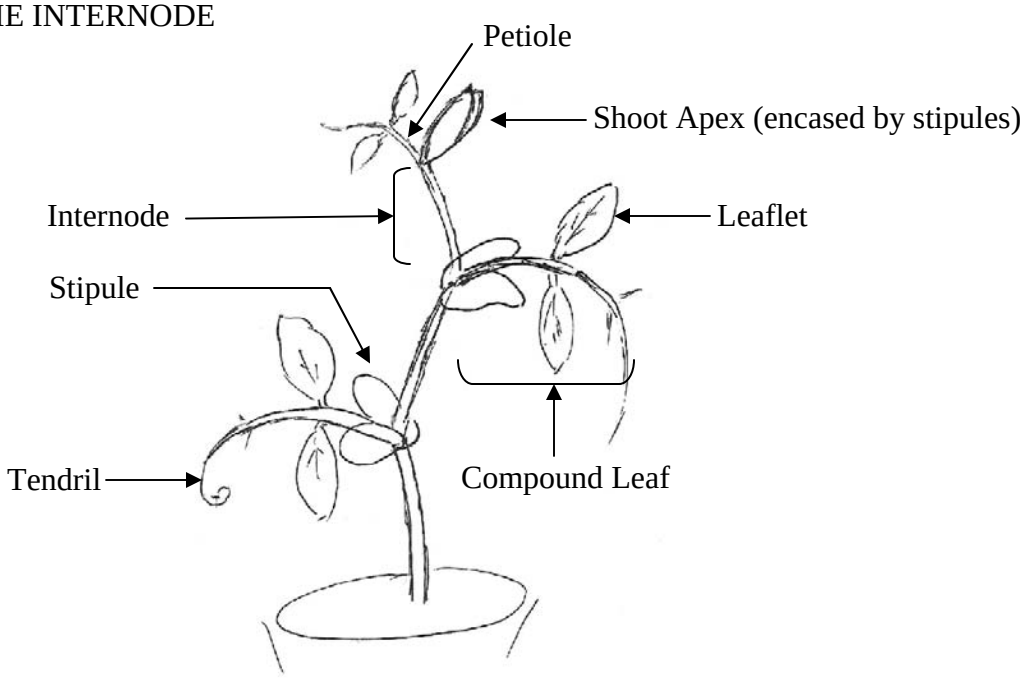
1. Each table has four pots each of '**Little Marvel'** and '**Alaska'** variety pea plants and will be responsible for the measurement of six plants in each pot.

2. If there are more plants than 6 per pot, remove the extras without disturbing the remaining plants.

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

Control (water plus tween-20)
1000ppm **GA** plus tween-20
1000ppm **Cytokinin** 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. REMEMBER, PEAS HAVE COMPOUND LEAVES…BE CAREFUL TO NOT CONFUSE THE PETIOLE WITH THE INTERNODE



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 both 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 Control, 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						
Average						

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						
Average						

Table C3. 'Little Marvel' variety results with Cytokinin 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						
Average						

Table C4. 'Alaska' variety results with Cytokinin 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						
Average						

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

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

Table C7. 'Little Marvel' variety results with B-NINE 1000ppm 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						
Average						

Table C8. 'Alaska' variety results with B-NINE 1000ppm 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						
Average						

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 compared to the control?

Did both varieties respond in the same way to GA?

How did cytokinin affect growth compared to the control and GA treatments?

How does the Control treatment compare to the GA treatment?

Is there a difference between the two internodes measured for any of the treatments?

Explain why any differences in the internodes might have occurred.

How did the varieties respond to B-Nine application compared to the control?

How do the B-Nine treatment compare to the GA treatments?

Formulate a hypothesis to explain your results?

D) Gravitropism vs. Phototropism (Work in groups of 4):

1. **Radish** seeds have been previously planted in centrifuge tubes containing vermiculite and grown under light. Select **Three** of the tubes and label them appropriately for identification by your group.

2. Each group should place a labeled germinated plant/tube in each of the following environments at the beginning of the lab period:

a) Dark/Horizontal
b) Top Light/Horizontal
c) Bottom Light/Horizontal

3. Be sure that the plants are oriented parallel to the surface they are placed on (the stem should be horizontal). Make drawings of the plants at the start as follows (you'll need this to compare with the final orientation):

Predict how the plant in the DARK/HORIZONTAL environment will respond:

Predict how the plant in the TOP LIGHT/HORIZONTAL environment will respond:

Predict how the plant in the BOTTOM LIGHT/HORIZONTAL environment will respond:

4. Observe the phenotype of the specimens at the end of the lab period. Notice any curvature, severity of curvature, or other developmental changes present.

How did the DARK/HORIZONTAL plant respond to the treatment?

How did the TOP LIGHT/HORIZONTAL plant respond to the treatment?

How did the BOTTOM LIGHT/HORIZONTAL plant respond to the treatment?

Indicate for each scenario, whether the tropic response was positive or negative.

Which tropism was dominant?

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