Name_____ Pre-Lab: Plant Structure and Growth

1. Explain the difference between determinate and indeterminate growth.

2. What regions in plants have the perpetual embryonic tissue that allows for continuous growth?

3. Graph the average leaf length with age. Put the values in the appropriate space on the axes. Connect the data points with a line.

	Length (mm)		
Age (days)	Leaf 1	Leaf 2	Leaf 3
0.5	5.0	4.0	3.0
3	4.0	4.0	4.0
7	6.0	11.0	11.0
14	98.0	83.0	92.0
21	121.5	148.5	137.5
28	143.5	139.0	136.0

Plant Structure and Growth

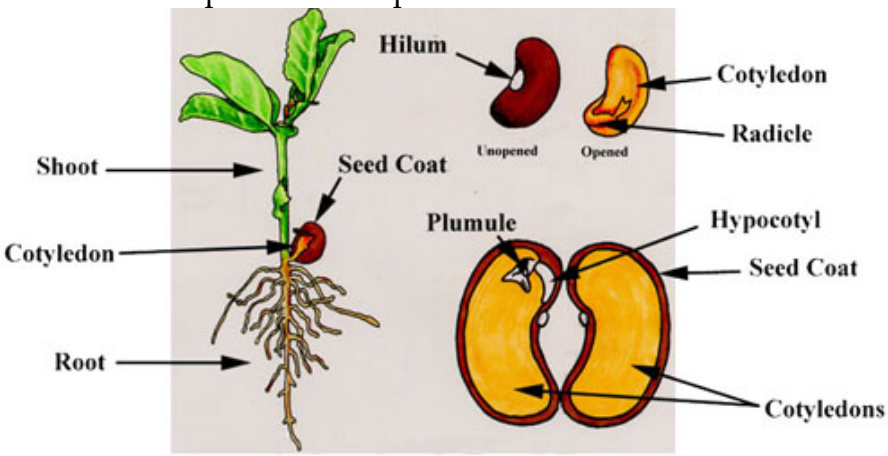
Purpose

Identify functional trends of plant structures with growth and development.
Distinguish between determinate and indeterminate growth.
Communicate observations graphically.

Introduction

From zygote to mature form, a plant develops through a series of changes involving both **growth** and **differentiation**. For any organism, the term growth refers to quantitative and irreversible changes that take place during the life cycle. (What is the fundamental process of growth in plants?) Differentiation applies to qualitative differences between cells, tissues, and organs that occur during the process of development.

A very young plant embryo consists of a spherical mass of rapidly dividing cells. However, when the embryo reaches a certain size, cell division becomes restricted to a few regions of the embryo, such as shoot and root meristems. Within an embryo, development proceeds by predictable and sequential steps that lead ultimately to the normal growth and development of the plant.



Cotyledons

A significant difference between plants and most animals is that plants have **indeterminate growth** while animals have **determinate growth**. Animals grow during a juvenile period until the adult form is reached and growth ceases. Cells are replenished and energy is generated by the organism but its size is limited. Plants, however, can grow in size throughout their lifespan. Regions in stems and roots have perpetually embryonic tissues called **apical meristems** and this is where primary growth occurs. Secondary growth appears in **lateral meristems** which causes thickness in woody plants. Certain parts of plants have determinate growth such as leaves,

flowers and fruit. In lab you will examine the growth trends of different plant structures through time. Consider the function of each structure and why it would nor would not be advantageous to have determinate or indeterminate growth. A lab write up is associated with this exercise, more detailed instructions will follow. Today you will:

1 – Dissect bean seeds to observe early growth stages.
2 – Observe and measure bean plants and their structures at various ages
3 - Determine leave surface area at various ages using digital imaging analysis

Materials

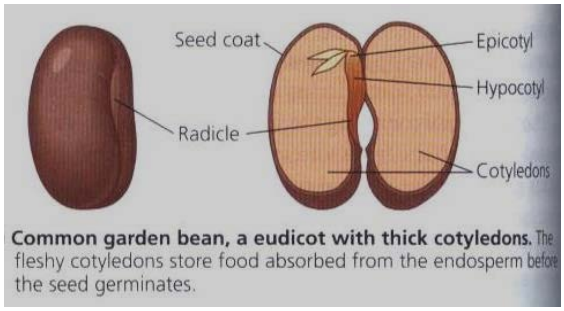
Bean plants (2 to 30 days old)
Large bucket of water
Large plastic beaker
Balance & weigh boats
Ruler (s)
Dissection scissors or blade
Paper towels
Camera or cell phone camera
Computer with Imag

How do juvenile bean plant structures differ from those found on older bean plants?

You will record your observations and measurements as described below, fill in the data tables at the end of the lab write up.

Part I. Early Growth

1. Select two bean seeds that have been soaking in water for several hours. Observe the scar on the seed coat, the **hilum**, where the seed was attached to the wall of the pod. Beside the hilum there is a small opening, the **micropyle**, through which the pollen tube enters the ovule. Remove the seed coat (testa) and notice the number of **cotyledons**. The embryonic axis lies between the two cotyledons. Locate the **hypocotyl** with the **plumule** (the parts that develop into the shoot and the first foliar leaves, respectively) and the **radicle** (the region that becomes the primary root).



Common garden bean, a eudicot with thick cotyledons. The fleshy cotyledons store food absorbed from the endosperm before the seed germinates.

   a) Sketch the bean seed and label the parts in bold above. Use the dissecting microscope to get a closer view.
   b) Measure the length of the first foliar embryonic leaves. Split them open and identify structures; label them on your drawings. Record the length in your data table.
2. Repeat this with beans which have been soaking for 1 day and 5 days.

Part II. Middle and Mature Growth

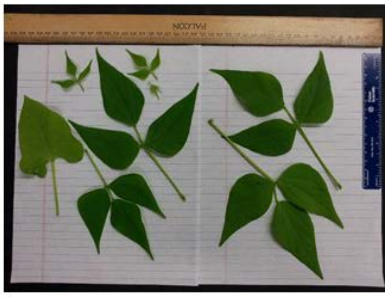
3. You will use two plants from each of the five age groups in the lab to make observations and measurements listed below. **To do this** you must rinse the soil away from the roots. Carefully remove each plant from its planter by submersing in a bucket of water, when the soil is saturated tip the planter and carefully remove the plant. Gently wave the roots around in the water to remove soil. Blot the roots dry and lay the plant down to take measurements. Carefully remove the leaves to measure, weight and photograph. **Do not** dump the soil and water down the drain!

Measurements:
Length of stem
Length of root
Number of nodes
Length of nodes
Leaf total weight
Root total weight
Leaf total surface area
Total leaf number
Number of Flowers
Length first foliar *leaves

Prepare plant: rinse roots

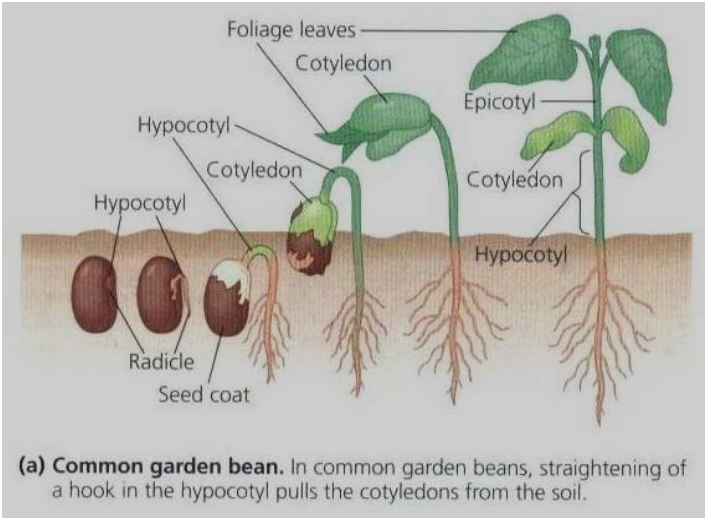
Measure stem & root length

Remove leaves, photograph, weigh & measure leaf length



To note:
*When measuring leaves measure from petiole base to the blade tip for the total length. List all measurements in your data tables.
Calculate the age of the plants you are measuring; the day each flat was planted is written on tape on the side of the plant box.

Procedure for Measurements of Plant Structures:
Work with one plant at a time. The idea is to get the full information for one plant to see growth through time; two plants per age will be averaged.
Place the flat in a bucket of water, until the soil is saturated, tilt the soil flat and gently lift your plant stem out by gently grasping at the base of the stem and separate the roots from the soil, trying to limit as much breakage as possible. Rinse in the large plastic beaker of water to



(a) Common garden bean. In common garden beans, straightening of a hook in the hypocotyl pulls the cotyledons from the soil.

further remove soil. Blot the entire stem and root system dry, cut where the where the stem turns completely white, below that is the root. With the plant carefully freed of soil, carry out the following:

a. What is the total length of the stem above ground?
b. What is the total length of the root system?
c. What is the length of the lateral roots closest to the surface of the soil?
d. Are cotyledons present? Record length in centimeters
e. Look at the stem, how many nodes exist?
f. Measure the node lengths. For each plant start with the first node, the hypocotyl, it is found between the cotyledon and the root. Node two is between node 1 and the first foliage leaves, node three is the next as you move towards the top of the plant.
g. Measure the total length of the first foliage leaves (lowest full leaf set) include the petiole in that measurement in centimeters. Then remove and set aside. Are any yellow?
h. How many additional leaves & leaflets does the plant have? Are any yellow? If so how many?
i. Are there flowers? If so how many? Which nodes have them?
j. Carefully remove and collect the leaves at the stem (so the leaf includes the petiole) keep the stem and leaves intact; use scissors if needed. Weigh the entire batch of leaves from one plant in a weigh boat and get total leaf weight in grams. Save leaves for the next step.
k. Arrange all leaves from one plant, flat on a white background with a ruler in view, a label as to the age of the plant and photograph all at once. The idea is to determine surface area so gently flatten the leaves to get the best representation of surface area in a photo. This can be measured later using the software ImageJ.
l. Measure total weight of roots in grams, be sure they are blotted dry.
m. Measure total weight of the stem in grams.
n. Remove and measure total weight of flowers.
o. Repeat with a second plant of the same age.
p. Repeat with all age groups.
q. Use ImageJ to determine area of photosynthetic structures (stem, leaves, petioles)

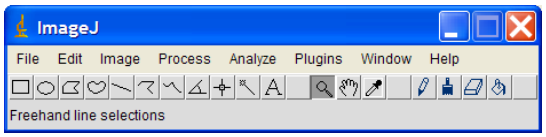
4. Examine Roots

a. Spend a few minutes observing the root structures of your bean plants.
Are there any noticeable patterns to the root growth?
Are there lateral roots?

b. Examine a prepared slide of a longitudinal section through an onion root tip or view the poster with the same image. The nuclei in these cells are stained and chromosomes in the nuclei can be seen in various stages of mitosis (use the 40x lens

to see this). Where mitosis is occurring constantly, new cells are being produced. This is the apical meristem. The root cap covers and protects the apical meristem from the soil. These cells are relatively larger and have less mitosis taking place. Behind the apical meristem is the zone of elongation. Here mitosis slows and the cells lengthen. Behind the zone of elongation is the zone of differentiation. Here the cells become specialized in structure and function. For example some cells develop into root hairs, others into transporting tissue such as xylem and phloem and others into additional types of cells. Make a labeled sketch below that includes the following: root cap, apical meristem, zone of cell elongation and zone of cell differentiation. Do you expect that roots would have determinate or indeterminate growth?

5. **Determine total leaf area per plant using ImageJ.** (*Free at http://imagej.nih.gov/ij/ to download ImageJ. Download and extract)*



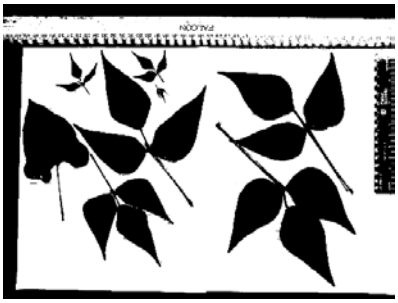
a. Carefully arrange and photograph all leaves from one plant at once, be sure to gently flatten the leaves, include a ruler in the photo to use in the measurement scale. Include the age of the plant in the name of your photo file and in the photo.

b. Open the program ImageJ Open imageJ

c. Go to file, open and find your image you want to work with

d. Go to *Image, type, 8bit*

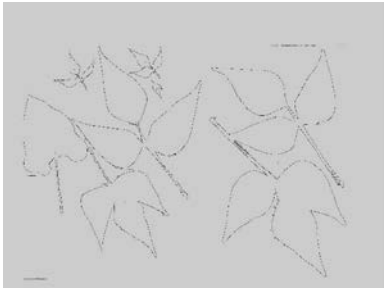
e. Set the scale by clicking on the line tool, draw a line along the ruler of known distance (10cm) and go to *Analyze, set scale*. A box opens:



f. Type in the known length (10) and add in the known units (cm) click OK

g. Double check by going to *Analyze, measure*
   a. It should say 10cm in the results for length

h. Go to *Process, binary, make binary* leaves and materials will be converted to high contrast and become black.



i. Use the rectangular tool to surround the entire area in which you want pixels counted and area calculated in cm^2. Click on it and draw around all **leaves**, but not anything else.

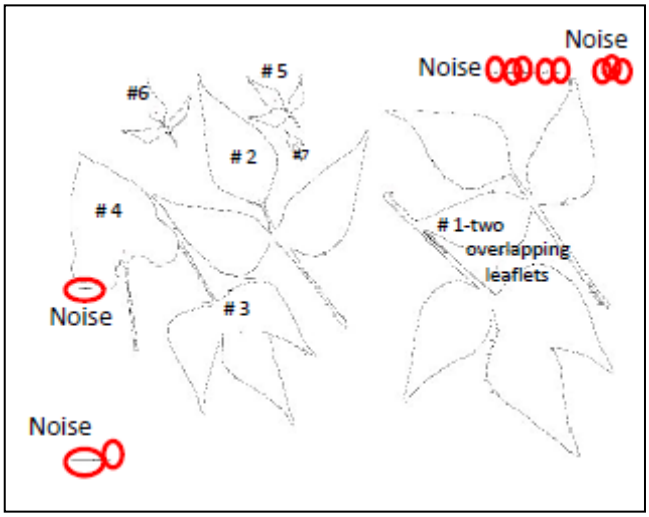
j. Go to *Analyze, particles*, enter 5 cm as the minimum particle size, Choose *"show outlines"*, check display

results, click OK

k. An outline of the analyzed area will be drawn and results will list each item it recognized and measured. Some will be artifacts, and very small.
l. Sum the area of the leaves do not include "noise".

The identified objects may be more than just your leaves, be sure to remove "noise" listed in the results, as seen in the example below.
It is the results of the image above to the right, created by ImageJ when calculating the surface area of the image it outlined above.
Example Results

Measures	Area **cm²**	Object
1	0.01	-noise
2	0.01	-noise
3	0.126	7
4	0.021	-noise
5	0.014	-noise
6	0.017	-noise
7	80.348	2
8	5.603	5
9	172.998	1
10	4.94	6
11	0.833	-noise
12	45.117	4
13	0.013	-noise
14	49.115	3
15	0.013	-noise
16	0.02	-noise
17	0.023	-noise
18	0.022	-noise
19	0.026	-noise
20	0.014	-noise
21	0.102	-noise
	359.385	



Total surface area – noise = surface area of the leaves
359.385 – 1.138 = 358.247 cm²

Lab assignments: Due in one week: graphs, drawings, a summary of your observations including answers to questions. Be sure to complete the following measurements and drawings by the end of lab today.
This is not a formal lab report, but will still take some time to produce properly.

Have the TA check off your raw data and drawings before you leave lab and time permitting draw simple graphs by hand to go over before you leave lab, to be sure you understand the proper depiction that you will produce and hand in next lab.

Questions:

- Why are we averaging the length of 2 leaves per plant, two plants per age? Why not just measure one leaf on one plant per age?
- Present your data graphically with the average measurements or counts on the y-axis and the leaf age in days on the x-axis as you did in the pre-lab, do it for each graph.
- Did you notice any interesting observations or trends with increasing age or the appearance of flowers?

In the summary of each graph include:

- Discussion of the nature of the curves obtained.
- Compare and contrast the growth of roots with the growth of leaves in terms of determinate versus indeterminate growth.
- Based on your graphic display of data, suggest which structures appear to have determinate growth or indeterminate growth.

Required:

Drawings of labeled dissected Bean seeds
Drawings of labeled root tip from the prepared slides or poster
Raw data tables
Written answers to all questions
The following five graphs and a written description of each

1. Average your data per age group
2. Plot the measurements of size v age for
    a. Root, stem, leaves, nodes
3. Plot the measurements of weight v age for
    a. root, stem, leaves, flowers
4. Plot the counts of structures v age for
    a. leaves, nodes, flowers
5. Plot total leaf surface area v age

Table 1. Various length, weight and counts of plant structures.					
Measurements		**5 days**	**10 days**	**20 days**	**30 days**
Length of stem (cm)	plant 1				
	plant 2				
	average				
Length of primary root (cm)	plant 1				
	plant 2				
	average				
Length of surface lateral root (cm)	plant 1				
	plant 2				
	average				
Stem total weight (g)	plant 1				
	plant 2				
	average				
Root total weight (g)	plant 1				
	plant 2				
	average				
Total leaf number	plant 1				
	plant 2				
	average				
Leaf total weight (g)	plant 1				
	plant 2				
	average				
Number of Flowers	plant 1				
	plant 2				
	average				
1st foliar leaf length (cm)	plant 1 leaf 1				
	plant 1 leaf 2				
	average				
	plant 2 leaf 1				
	plant 2 leaf 2				
	average				
Total leaf surface area (cm^2)	plant 1				
	plant 2				
	average				
# of yellow leaves	plant 1				
	plant 2				
	average				

Table 2. Node count and length					
Measure		**5 days**	**10 days**	**20 days**	**30 days**
Number of nodes	plant 1				
	plant 2				
	average				
Length of node	Plant 1 Node 1				
	Plant 2 Node 1				
	average				
	Plant 1 Node 2				
	Plant 2 Node 2				
	average				
	Plant 1 Node 3				
	Plant 2 Node 3				
	average				
	Plant 1 Node 4				
	Plant 2 Node 4				
	average				
	Plant 1 Node 5				
	Plant 2 Node 5				
	average				
	Plant 1 Node 6				
	Plant 2 Node 6				
	average				
	Plant 1 Node 7				
	Plant 2 Node 7				
	average				
	Plant 1 Node 8				
	Plant 2 Node 8				
	average				
	Plant 1 Node 9				
	Plant 2 Node 9				
	average				
	Plant 1 Node 10				
	Plant 2 Node 10				
	average				

Table 3. **Additional observations**					
		5 days	**10 days**	**20 days**	**30 days**