Chapter 14

Light-Induced Phenotypic Plasticity in Plants

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Contents

Introduction

The purpose of this exercise is to evaluate the phenotypic plasticity shown in response to differing levels of light intensity by two plant species. The plant material used in is produced by clonal propagation so that differences observed under high and low light levels are the result of phenotypic, not genotypic variation. Because the course emphasizes an evolutionary approach, two species, predicted to differ in degree of plasticity, are compared. In this exercise students will learn to make quantitative observations of leaf size and chlorophyll content by weighing and measuring leaves and by using a compound microscope and spectrophotometer. Statistical analysis of the null hypotheses is carried out by *t*-tests. The objects of the exercise are:

1. To test whether certain aspects of leaf size and chlorophyll content of plants are changed in response to growth in different light intensities.
2. To determine whether two species, *Impatiens parviflora* and *Pothos aureus*, differ in the degree of response to different light intensities.

This exercise normally requires approximately 3 hours to complete. Most of the time is needed for the chlorophyll extraction and to make and observe leaf cross-sections. In terms of level of difficulty, students would be at an advantage if they came to the lab with a general knowledge of the operation of compound microscope, and Student's *t*-test. However, this is not absolutely necessary and they will learn these techniques, as well as operation of a spectrophotometer and preparation of leaf cross-sections, by the end of the exercises. Experience with chlorophyll extraction has taught us the need for the students to be extremely careful when doing the extraction to avoid spills, cross contamination of extracts, and careful measurements of volume and absorption.

With the exception of the chlorophyll extraction, students work individually and each person will collect measurements from a single plant. Encourage cooperation between lab partners since they will share the same species (but different light regime). Groups of four at a bench will cover both species and both light regimes, all four treatments. Students must use only fully expanded leaves that grew right from inception under the light treatments for *all* measurements.

Materials

You will need the following materials for each group of four students:

Metric ruler, 15 cm (4)
Scissors (4)
Marker, to make labels (1)
Pencil (4)
Blank paper, 8.5" × 11" (4 sheets)
Metric graph paper, 8.5" × 11" (1 sheet)
Kimwipes (1 box)
Paper towel (10 sheets)
Mortar and pestle (2)
Graduated cylinder, 10 ml (2)
Petri dish with lid (2)
Razor blade, double-edge (snap in half before unwrapping) (4)
Plastic funnel, small (2)
Watch glass (12)
Tap water, in dropping bottle (1)
Glass microscope slides and cover slips (8)
Dissecting needle (4)
Paint brush, fine (4)
Whatmann filter disk, 11 cm (4)
Spectrophotometer cuvette (1)
Glass vial (such as a liquid scintillation vial), 20 ml (8)
Ice bucket (opaque), with ice (1)
Parafilm, small squares (12)
Methanol, 90% (250 ml)
Tabletop spectrophotometer (1)
Top loading balance (1)
Impatiens grown under high light (1)
Impatiens grown under low light (1)
Pothos grown under high light (1)
Pothos grown under low light (1)

Notes for the Instructor

This exercise was initially designed to illustrate interspecific differences in *phenotypic plasticity*, defined as "the ability of an organism to change its morphological and/or physiological features after exposure to different environmental conditions" (Bradshaw, 1965; Schlichting, 1986; Thompson, 1991). Phenotypic plasticity is thought to be an adaptive trait that is under genetic control, and both interpopulation and interspecific differences have been shown in degree of plasticity expressed under common garden or controlled growth conditions (Cook and Johnson, 1967; Thompson, 1991). The difference between the genetic and phenotypically plastic components of intrapopulation variation and the relative contributions of the two kinds of factors provide a focus for discussion related to this exercise.

In an evolutionary and ecological context, it is useful to consider the time scale of environmental change in relation to the adaptive responses of plants and animals (Osmond and Chow, 1988). When change occurs over millennia or centuries (such as a significant climatic change), new conditions will select for certain genotypes and evolutionary change will occur with successive generations. When changes occur over days, weeks, or months (such as neighbouring tree fall, creating a light gap in the canopy) an individual plant that is able to alter certain features so that it can function better under the new conditions is more likely to survive and reproduce. When environmental changes occur over much shorter time periods (such as a sunfleck that moves across the forest floor) plant response is usually at the subcellular level and involves the light reaction centers of photosynthesis (Bjorkman and Holmgren, 1963).

This exercise makes use of the structural and pigment characteristics of leaves that have developed over a 6- to 9-week time period under two different light intensities (irradiance levels); growth conditions are discussed in Appendix B. Two readily obtainable horticultural species are used: *Pothos aureus*, representative of a slow growing species adapted to the stable and dimly lit conditions of the rain forest understorey, and *Impatiens parviflora*, representative of a faster

growing "weedy" species that establishes itself in light gaps under tropical conditions. We initially predicted that *Impatiens* would show a greater degree of plasticity than *Pothos*, but the species' response is more complex than this. *Pothos* is slower growing, "invests" more resources in each leaf, and responds to different light levels by changes in chlorophyll content (and presumably other subcellular changes). *Impatiens* produces leaves more rapidly, and new leaves show a number of structural changes with little change in chlorophyll content.

Besides the features measured in this exercise, other features of these plants may be investigated for phenotypic change, including internode length, stem diameter, leaf anatomy, and chlorophyll *a*/*b* ratio. Leong and Anderson (1984) found that the ratio of chlorophyll *a* to *b* increases when plants are grown in high light intensity. This reflects a change in the stoichiometric relationships between reaction centers of higher plant photosystems and their associated light harvesting antenna pigment complexes. Reaction centers contain only chlorophyll *a*. Chlorophyll *b* is present exclusively in the group of peripheral protein pigment molecules attached to these photosystems. Thus, a decrease in the chlorophyll *a*/*b* ratio indicates the enrichment of light harvesting chlorophyll antenna proteins relative to the reaction centers. For more reading on this consult Anderson (1986). Another focus for discussion would be the selection of more suitable experimental material (e.g., related species of the same genus).

The method provided in this exercise for making cross-sections works well, but there are at least two other ways of preparing them: (1) sandwiching the leaf piece between two pieces of carrot or potato and drawing the razor blade towards you, or (2) placing the leaf piece in a drop of water on a microscope slide and then rapidly cutting or "dicing" the leaf to create thin cross-sections. Note that we provide illustrations of digital and analog spectrophotometers the in the outline that we distribute to our students (these are not provided in this chapter.)

Student Outline

Introduction

If environmental conditions become unfavourable for the survival of an organism, it normally has two options: either to move or change in a way that allows it to function under the new conditions. For plants, which are nonmotile, the only alternative is to adjust to the new conditions, usually by an alteration of growth pattern or modification of subcellular machinery. The ability of an individual to change its morphological and/or physiological features after exposure to different environmental conditions is known as phenotypic plasticity.

As an example, a single individual of the aquatic buttercup, *Ranunculus tripartitus*, can produce two different types of leaves: submerged and floating (Sculthorpe, 1967). Submerged leaves have long, thin lobes and are adapted to carry out photosynthesis in the reduced light levels underwater. Floating leaves, on the other hand, have a broad surface area and features that suit photosynthesis under higher light levels at the water surface.

Phenotypic plasticity is an important adaptation that can optimize plant survival and therefore extend the geographic range over which they grow. Plant species differ in the degree of phenotypic plasticity displayed. Plants growing in stable environments are less likely to show morphological and/or physiological variability, while plants growing in unstable environments often are phenotypically plastic. Common examples of unstable environments are shallow ponds or gaps in the rain forest canopy. In these cases, changes in water or light level occur over days or weeks and evolutionary change, where new genotypes are selected by the new conditions is too slow. A plant is more likely to survive and reproduce in an unstable environment if an individual can modify itself morphologically and physiologically.

Since photosynthesis is the sole source of energy for most plants, light is one of the key requirements for survival and growth. Capture of the optimum amount of light, especially in the face of competition from other plants, is extremely important. It is not surprising then that plants from environments with fluctuating light levels have evolved high levels of phenotypic plasticity in response to changes in light intensity. In this laboratory you will be investigating the responses shown by two species of plants, *Impatiens parviflora* and *Pothos aureus* (see Figure 14.1), when grown under two different light intensities.

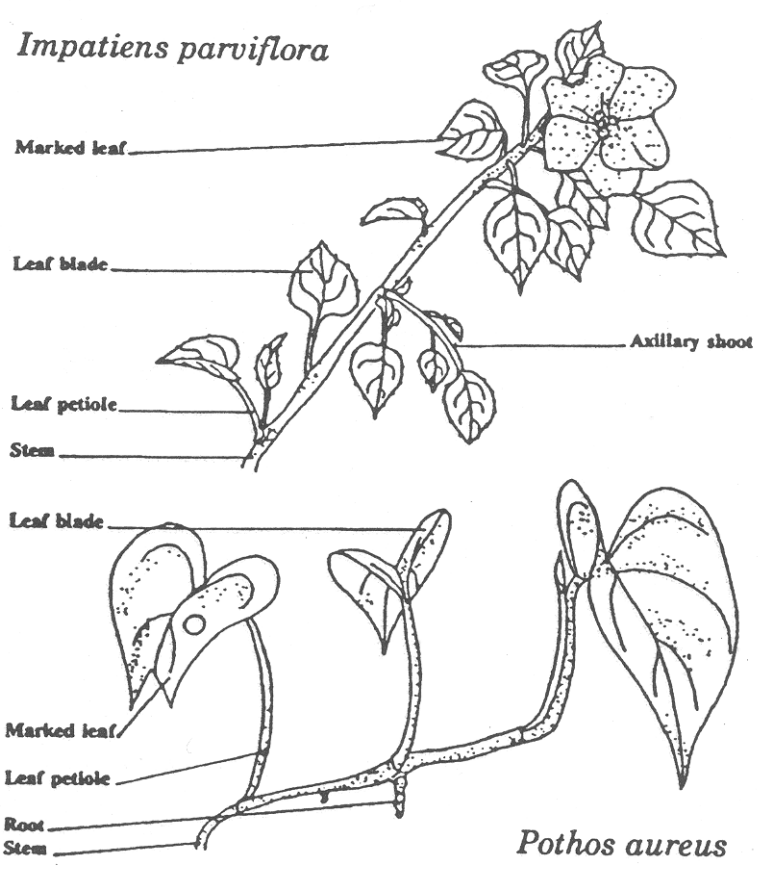

Figure 14.1. *Impatiens parviflora* and *Pothos aureus*.

Pothos is a climbing plant from the dimly lit rain forest understorey of the tropical Solomon Islands, while *Impatiens* is a weedy plant that colonizes newly-formed light gaps in the tropical forests of East Africa. The characters you will measure or observe are Specific Leaf Weight (SLW, a weight per unit area), petiole length (important in preventing shading from adjacent leaves), leaf thickness, and chlorophyll content per unit leaf area. Leaves are the most important site of photosynthesis and all of these features have been shown to be strongly correlated with photosynthetic rate.

Procedures

Leaf Weight

Locate the leaf on your plant which has been marked in a particular way. This leaf formed after the start of the experiment and leaves nearer the tip have all developed under the experimental light regime. Use one of these leaves for your measurements.

Select and remove, at the base of the petiole (i.e., where it joins the main shoot), one fully expanded leaf that has formed during the experimental period. Remove the petiole from the blade (do *not* discard it). Weigh the blade (in mg) and enter the value in Table 14.1; obtain data from your neighbour to also add to your data table.

Table 14.1. Measurements of leaf fresh weight and area for *individual* data.

	Plant species:		
	Leaf fresh weight (mg)	Leaf area (mm^2)	SLW (mg/mm^2)
Low light			
High light			

Leaf Area

Measure the area of your leaf as follows: Trace the outline of the leaf on blank paper. Cut out the shape and weigh it on a balance. Using a piece of paper and your ruler, cut out and weigh a piece of the same paper of known area (e.g., 40 mm × 40 mm). By proportional calculation, determine the area (in mm^2) for the leaf. Record your data and your neighbour's data in Table 14.1.

Specific Leaf Weight

Calculate a specific leaf weight (SLW) for your and your neighbour's leaves (use the individual values for area and weight for each leaf to calculate the SLW) using the formula shown below. Add your values to the class data table at the front of the room and complete Table 14.2.

$$SLW = \frac{leaf\ weight\ (in\ mg)}{leaf\ area\ (in\ mm^2)}$$

Petiole Length

Measure the petiole length (in mm) for your leaf and record it in Table 14.3. Enter your value for petiole length in the class data table at the front of the room and record the class data in Table 14.4.

Table 14.2. *Class* data for SLW.

	Light intensity	SLW (mg/mm^2)		
			s	N
Impatiens	Low			
	High			
Pothos	Low			
	High			

Table 14.3. Petiole length for *individual* data.

	Species:
	Petiole length (mm)
Low light	
High light	

Table 14.4. *Class* data for petiole length.

	Light intensity	Petiole length (mm)		
			s	N
Impatiens	Low			
	High			
Pothos	Low			
	High			

Preparation of Leaf Sections and Measurement of Leaf Thickness

1. Prepare hand sections of your leaf using the following technique (see Figure 14.2). Remove a piece of leaf blade 1 cm × 2 cm and fold in half longitudinally. Wet both surfaces of the leaf and the edge of a razor blade in tap water.
2. Hold the leaf firmly in a vertical position and cut sections by slicing slowly and deliberately toward you. Complete cross-sections are often not necessary. You may find it easier to cut thin sections by resting the blade on one side of the cut surface and making smaller wedge-shaped sections by exerting pressure downwards as you slice horizontally. Ideally, sections should be less than the average diameter of a parenchyma cell (approximately 25 μm).
3. Use a fine paintbrush to transfer the sections from the razor blade to a watch glass filled with tap water. Gently move the sections to remove the debris from the cut cells.
4. Transfer sections to tap water in another watch glass. Rinse by moving gently for about 1 minute.
5. Mount sections in a drop of tap water on a microscope slide. Lower one side of a coverslip gently with a dissecting needle so that air bubbles are not trapped.
6. Scan the slide at low power to locate the specimen. Determine the orientation of the specimen and identify which is the upper surface of the leaf (i.e., direction of light); look for the elongate palisade cells that are always formed towards the upper surface. Exchange slides with your neighbour, and study the leaf anatomy of the same species grown under a different light regime. (Consult Appendix C for assistance). What differences can you see? Record the features in

Table 14.5. (Since you will be using ocular micrometers to measure leaf thickness, calibrations are provided in Table 14.6.)

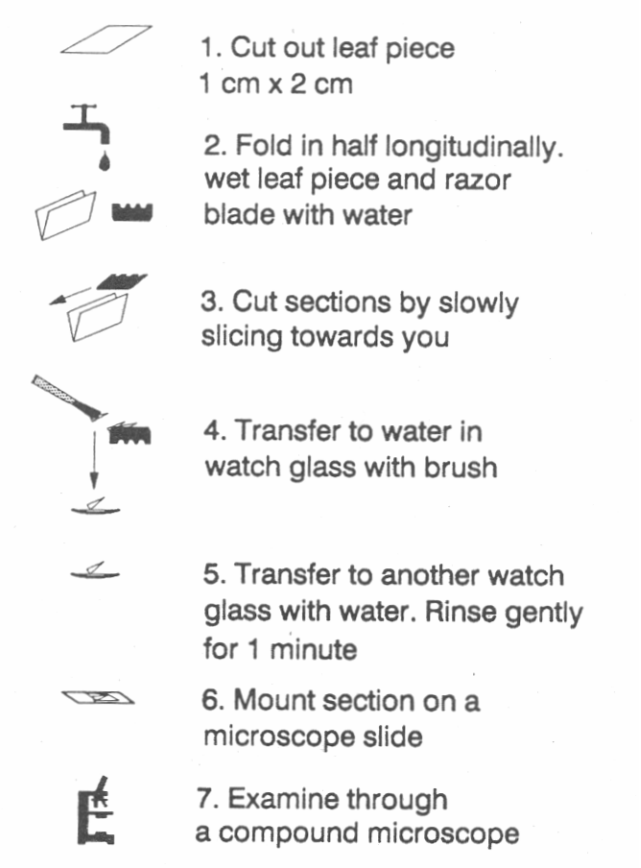

Figure 14.2. Flowchart for preparation of leaf cross-sections of *Impatiens* and *Pothos*.

Table 14.5. Differences in leaf anatomy.

	Species:	
	Low light	High light
Anatomical differences		
Leaf thickness (μm)		

Table 14.6. Calibration of an ocular micrometer at different microscope objectives.

Power	1 unit of ocular scale =	
	Monocular microscope	Binocular microscope
low	(4X) 26.5 µm	(3.2X) 32.3 µm
10X	10.5 µm	10.5 µm
40X	2.5 µm	2.5 µm

7. Measure the *maximum* thickness (in µm) of your leaf cross-section. If there is a vein in your preparation, do not measure thickness at this position. Measure from the outside of one epidermis to the outside of the other. Record your leaf thickness, and that of your neighbour's in Table 14.5. Combine your results with those of others in the room and enter the mean, standard deviation and sample size in Table 14.7.

Table 14.7. *Class* data for leaf thickness.

	Light intensity	Leaf thickness (µm)		
			s	N
Impatiens	Low			
	High			
Pothos	Low			
	High			

Chlorophyll Extraction

Before you begin, remember that whenever you insert a spectrophotometer cuvette into a spectrophotometer make sure you match up the line on the cuvette with the one on the sample holder. By doing this you will ensure that the analyzer in the spectrophotometer will always read through the same area of glass on the cuvette. The first thing to do is to turn on the spectrophotometer to let it warm up for at least 15 minutes. Do this by turning the Power Switch/Zero Control Knob clockwise.

For chlorophyll measurements it is best to work as a group. Given below are detailed instructions on the extraction of chlorophyll from leaf pieces as adapted from Arnon (1949). Figure 14.3 presents the same protocol in a flowchart format.

1. Cut two 1-cm^2 squares (or 1 piece of 2 cm^2 area) out of the center of a new leaf (i.e., a leaf that developed under the experimental conditions) from each treatment using a piece of graph paper as a guide. Try not to include the midvein and do not forget to use only leaves formed during the light treatment.

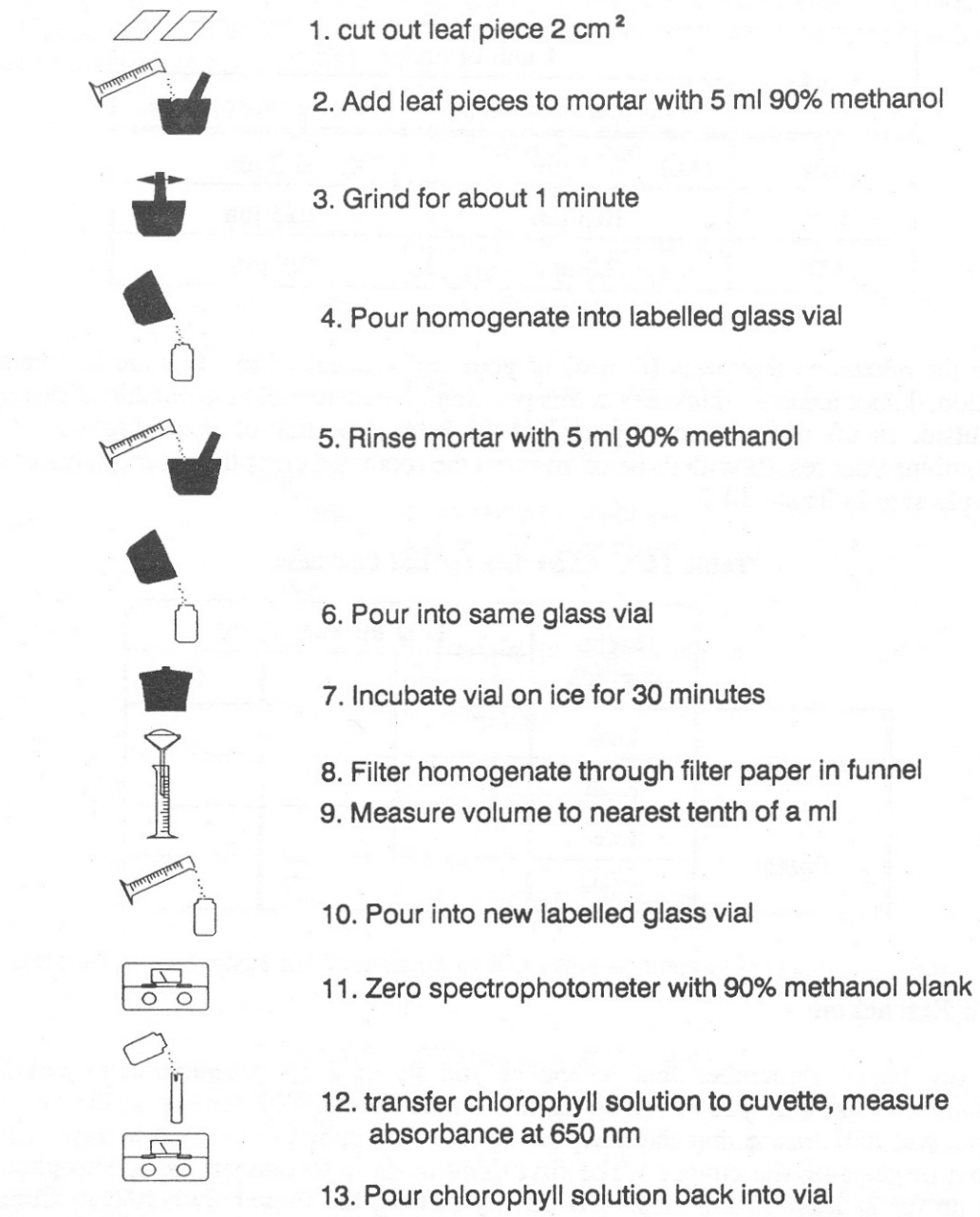

Figure 14.3. Flow chart for of chlorophyll from *Impatiens* or *Pothos* leaf pieces.

2. Add the leaf squares and 5 ml (measured in a graduated cylinder) of 90% methanol to a mortar and pestle, and carefully grind the squares to a homogeneous pulp (make sure there are no large, green leaf fragments remaining). Grind for about 1 minute, especially for *Pothos*.

3. Pour the resulting homogenate into a glass vial labeled with the species of plant and the light treatment. Add another 5 ml of methanol to the pestle and, after gently swirling to collect the remaining leaf material, pour the solution into the same vial.

4. Cover the vial with parafilm and place it in an ice bucket for 30 minutes. Cover the bucket with the lid. Complete steps 2, 3, and 4 for all four leaf pieces. Clean the mortar and pestle with a paper towel and a small amount of 90% methanol between extractions.

5. After 30 minutes, remove one of the sample vials from the ice bucket and filter the homogenate through filter paper into a graduated cylinder and measure the volume to the nearest 0.10 ml. Record this volume in Table 14.8. Pour the solution into a new vial labeled in the same way as the original.

6. Zero the spectrophotometer using a blank solution of 90% methanol in your marked cuvette (the cuvette must be at least half full to make a measurement possible in the spectrophotometer). First set the Wavelength Control Knob (on the upper right hand side) to 650 nm. Empty the 90% methanol into a clean vial (labeled "blank") and cover with parafilm. Before placing the 90% methanol blank into the chamber adjust the Power Switch/Zero Control Knob until the needle on the display reads infinite absorbance (or zero transmittance).

   Wipe the sides of your blanking cuvette with a kimwipe and insert it into the sample chamber. Line up the mark on the cuvette with the one on the sample holder. Adjust the meter to read zero absorbance with the Light Control Knob (located on the lower right hand side). Remove the blank, the machine is now zeroed. Tap out as much of the excess 90% methanol from your cuvette as possible.

7. Pour the filtered chlorophyll solution from the vial into the cuvette. Place the cuvette back into the sample holder and close the lid. Measure the absorbance of the solution directly from the meter. Record this value in Table 14.8. Pour the chlorophyll solution back into the vial. Rinse out your cuvette using two quick rinses of 90% methanol. Discard waste 90% methanol. Repeat steps 5 and 7 for all four chlorophyll samples. Rinse between measurements to keep contamination to a minimum. Repeat steps 5, 6, and 7 exactly as described above, except that the wavelength used for zeroing and measuring absorbance is 665 nm. Do not forget to record the absorbance value in Table 14.8. Two wavelengths are used because we want to measure the amounts of chlorophyll *a* and *b* and 650 and 665 nm, respectively, are specific for these types of chlorophyll.

8. Calculate the amount of chlorophyll (in µg) present in each of the vials using the formula:

$$[25.5\,(O.D._{650}) + 4.0\,(O.D._{665})] \_ ml\ of\ methanol = \mu g\ of\ chlorophyll$$

9. The amount of chlorophyll thus calculated can be expressed on a per cm^2 basis — remember that you extracted from 2-cm^2 of leaf area. Enter the chlorophyll content for each treatment in Table 14.8 and in the class data table at the front of the room. You can then combine all chlorophyll data from the rest of the students conducting the experiment and record the results in Table 14.9.

Table 14.8. *Individual* data for chlorophyll content.

	Light intensity	Chlorophyll content (cm^{-2})			
		Amount of extraction solution (ml)	Absorbance values		Chlorophyll content ($\mu g\ cm^{-2}$)
			650 nm	665 nm	
Impatiens	Low				
	High				
Pothos	Low				
	High				

Table 14.9. *Class* data for chlorophyll content.

	Light intensity	Leaf thickness (µm)		
			s	N
Impatiens	Low			
	High			
Pothos	Low			
	High			

Interpretation of Results

Now that you have your results, you are ready to statistically test whether the differences in leaf morphology that you observed under the different light intensities are large enough to actually reflect phenotypic plasticity, and not just sample error. At this point you should remind yourself what are the hypotheses you are testing. The null hypotheses stated below are for *Impatiens*; the same hypotheses can also be asked for *Pothos*.

Specific Leaf Weight: There is no significant difference in mean SLW between leaves grown under two different light intensities for *Impatiens*.

Petiole length: There is no significant difference in mean petiole length between leaves grown under two different light intensities for *Impatiens*.

Leaf thickness: There is no significant difference in mean leaf thickness between leaves grown under two different light intensities for *Impatiens*.

Chlorophyll content: There is no significant difference in mean chlorophyll content between leaves grown under two different light intensities for *Impatiens*.

To test your hypotheses about the differences between plants of a given species grown under the different light intensities you will be using Student's *t*-test. Conduct the eight *t*-tests to evaluate all the hypotheses (four comparisons for each of two species). Enter the results in Tables 14.10 and 14.11.

Table 14.10. Results of *t*-tests for *Impatiens parviflora.*

	Observed *t*-value	Degrees of freedom (*df*)	Critical *t*-value	Accept or reject H_0?
SLW				
Petiole length				
Leaf thickness				
Chlorophyll content				

Table 14.11. Results of *t*-tests for *Pothos aureus*.

	Observed *t*-value	Degrees of freedom (*df*)	Critical *t*-value	Accept or reject H_0?
SLW				
Petiole length				
Leaf thickness				
Chlorophyll content				

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APPENDIX A
Student's t-test

With equal sample sizes, the formula for the Student's *t*-test can be expressed as:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_1}}}$$

With unequal sample sizes, the formula is written:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1 - 1)\, s_1^2 + (n_2 - 1)\, s_2^2}{n_1 + n_2 - 2}\left(\frac{n_1 + n_2}{n_1 \_ n_2}\right)}}$$

APPENDIX B
Growth of Plant Material

Both *Impatiens* and *Pothos* can be grown from cuttings and are quite amenable to most potting soils. Typically one 7.5-cm plastic pot per plant is sufficient. Immediately after placing the plants under the experimental light conditions, the top-most leaf should be marked with either a holepunch or with indelible paint. This will serve to mark the last leaf formed outside of the experimental conditions. *All leaves formed after this marked leaf will have grown under the correct light intensity. Students should use only these leaves for their measurements.*

Pothos and *Impatiens* plants are grown for a number of weeks in either high light or low light, while other environmental variables (e.g., temperature and relative humidity) are held constant so that their effects will not confound those induced by the different light levels. Light intensities normally used are 30 $\mu Em^{-2}s^{-1}$ (μE = microeinsteins) for the low light treatment and 150 $\mu Em^{-2}s^{-1}$ for the high. Intensities are measured using a radiometer. Pilot experiments have shown that, if need be, extreme morphological differences can be induced by using 15 and 300 $\mu Em^{-2}s^{-1}$ for the low and high light treatments, respectively. To avoid increased variability due to differences in light intensity at different places under light sources, plants should by periodically rotated and repositioned under the lights.

The experimental plants will require about 7 to 8 weeks under the light conditions (at 22°C) to show the appropriate responses. If you are going to use a lower temperature, a longer treatment period is necessary, for example 9 or 10 weeks at 20°C. Treatments longer than required will not harm the plants. It is important to keep the plants from mutually shading each other as much as possible as this will tend to produce variable results. *Pothos* is quite hardy and will require minimal care. *Impatiens*, especially those under the high light treatment can dry out and will need to be watered more frequently.

APPENDIX C
Leaf Anatomy

Use the anatomical diagrams provided in Figure 14.4 to help identify portions of the anatomy of *Impatiens* and *Pothos* leaves. The *upper epidermis* (UE) is a continuous layer of transparent cells; only a small amount of light is absorbed when shining through this layer. You may be able to recognize the layer of cuticle which retards water loss from the leaf surface. The *palisade mesophyll* (PM) layer, directly beneath the upper epidermis, is composed of elongate sausage-shaped cells that carry out most of the photosynthesis; depending on the light treatment there may be 1, 2, or more layers. It is thought that one reason for the elongate shape is that with cytoplasmic streaming the cytoplasm is stirred and the chloroplasts first come up into the direct light and then move down to the more shaded part of cell so that no chloroplast becomes overheated. Below are the photosynthetic *spongy mesophyll* (SM) cells; usually more air space is visible between the cells in this layer, air space which is in direct contact with the stomata (S). In most leaves, the *lower epidermis* (LE) contains most of the stomata. Each stomata is composed of a pair of guard cells. Veins or vascular bundles present in the leaves contain xylem which carries water to the photosynthetic mesophyll cells and phloem which takes the carbohydrates produced by photosynthesis in the mesophyll and transports it to the rest of the plant.

Unique features to look for are the presence of specialized *secretory cells*: mucilage (M), tannin (T), and crystal (C) cells. The presence of these cells is evidence of a healthy plant; because healthy plants have high rates of photosynthesis it is as if they have carbon reserves left over where they can make these specialized compounds, which are thought to act as deterrents to fungal growth and to feeding insects and herbivores.

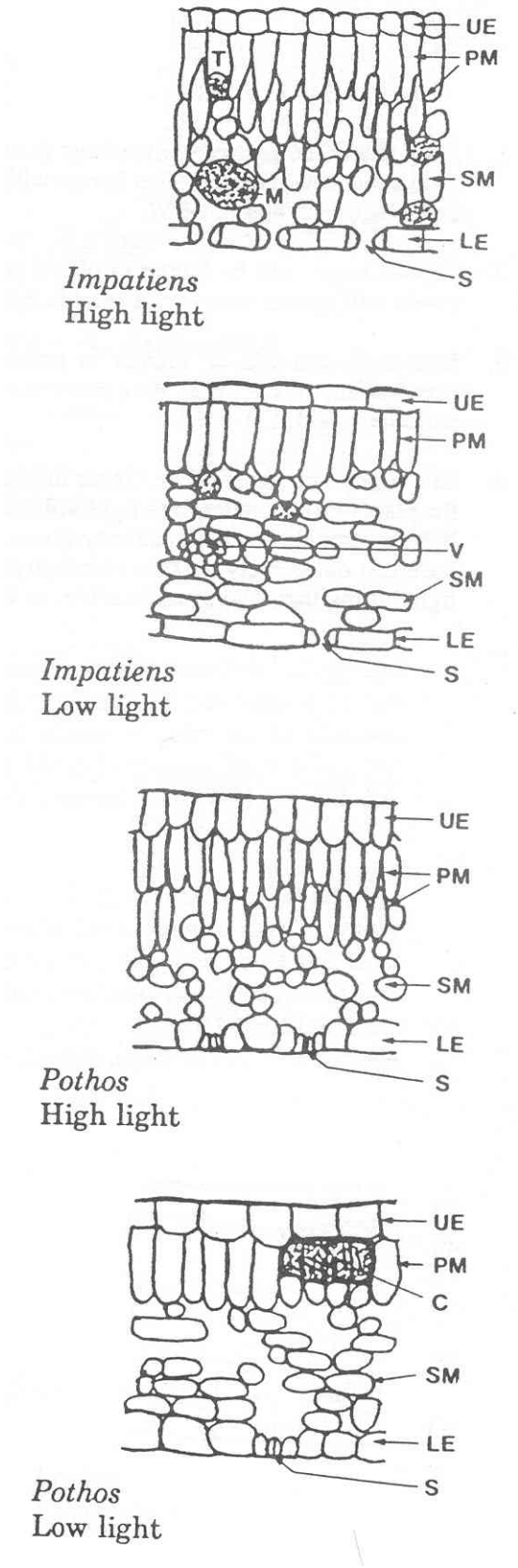

Figure 14.4. Diagrammatic cross-sections of *Pothos* and *Impatiens* leaves grown under two different light intensities

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APPENDIX D
Expected Results

1. SLW should be greater in the plants grown under high light since SLW measures leaf density. High light grown plants will have thicker leaves with more photosynthate (starch) and thicker cell walls. These factors contribute to a higher SLW.

2. Petiole length will be longer in plants grown under low light. Due to the low availability of light, these plants will spread their leaves out to capture as much of the light as possible and to avoid mutual shading.

3. Leaf thickness will be greater in plants grown under high light. High light intensities mean greater penetrability of the light. As a result leaves can be thicker and still have cells on the lower side receiving sufficient light.

4. Chlorophyll content will be higher in plants grown under low light. Although this seems counterintuitive, the plants grown under high light voluntarily limit their chlorophyll due to the fact that they have enough light to provide for their photosynthetic needs with a limited amount of chlorophyll and that too much light can oxidatively destroy chlorophyll. Plants grown under low light need to capture as much of the light hitting their leaves as possible, so they increase the density of chlorophyll molecules in their leaves.