Plant-A-Plant Carbon Dioxide Laboratory Guide

Task

Transplant maize seedlings into a closed system (plastic bottle) in order to monitor the effect of decreased carbon dioxide (CO_2) concentration on plant growth. This decreased level of CO_2 will be caused by the presence of sodium hydroxide, which absorbs CO_2 from the air (reacting with carbon dioxide to form sodium carbonate and water $2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$). After 14 days of cultivation, harvest plants, make calculations and compare the results between treatments.

Pre-Lab Instructions

1. In your lab groups, begin by considering the following lab questions. In your lab notebook brainstorm your initial ideas possible answers and/or how you might use an experiment to find the answers.
2. Read the lab guide and discuss the experimental design. How does the design test for the carbon dioxide requirements of plants?
3. Develop and record an experimental hypothesis in your lab notebook.
4. Based on your discussions and lab procedures, determine the location for your experiment. Record in your lab notebooks why you chose the location and describe how conditions are suitable for individual parts of the experiment.
5. Develop a group schedule for plant cultivation, daily watering responsibilities, making and recording observations, etc.

Lab Questions

How does a lower concentration of CO_2 in the air affect plant growth?
Is CO_2 a limiting factor to growth?
Hoes does CO_2 from the air get into plant leaves?
How is CO_2 stored in plants?
Hoes does increased CO_2 in the air affect plant growth and carbon storage?

Prepare and Perform the Experiment

Materials and Tools (*per replicate*)

- ❑ 12 maize seedlings
- ❑ 2 clear plastic bottles (at least 1 liter) to create closed system for plants
- ❑ Fertilizer containing basic nutritients (for example, Kristalon Start or Miracle Gro)
- ❑ Distilled water (1 liter)
- ❑ Measuring cylinder
- ❑ Laboratory scale (accuracy of 0.01 gram)
- ❑ 1 beaker or glass jar (volume 100 ml)
- ❑ Razor blade or sharp scalpel
- ❑ Aluminium foil
- ❑ Scissors
- ❑ Oven
- ❑ Test-tube or a small bottle (volume 10-15 ml)
- ❑ Tweezers/forcepts

- ❑ Gloves & Safety goggles/glasses
- ❑ Sodium hydroxide (NaOH)
- ❑ Pencil, permanent marker
- ❑ Scotchtape (cellotape) for labeling
- ❑ *Laboratory Data Sheet* – Carbon Dioxide
- ❑ *Student Laboratory Questions Sheet*

** Note: At least two replicates are recommended for this experiment.

Preparation

1. If necessary, calculate the amount of materials needed for more than 1 replicate.

2. Wash the bottles thoroughly - do not use any cleansers, since these could influence the growth of plants. Allow the bottles to air-dry. Be sure to choose transparent bottles, since colored plastic will affect the growth of the plants.

3. Cut the top 1/3 off the bottles using scissors or a razor blade. Be careful as you will need to tape the top back to seal the bottle on after you plant the seedlings and add the sodium hydroxide.

4. Mix a 0.2 g/l fertilizer solution. Weigh 0.2 g of fertilizer and dissolve into one liter of distilled water. Pour fertilizer solution into a labeled bottle. The fertilizer solution will be the growth medium for the CO2 experiment.

5. Add 50 ml of fertilizer solution to each experimental bottle.

Prepare Seedlings

Note: The influence of carbon dioxide concentration on plant growth will be more pronounced if the seedlings are modified to depend entirely on photosynthesis rather than stored compounds. Therefore, you will remove the majority of the endosperm of the germinated seed (the part of the seed that stores compounds for growth), careful not to damage the remainder of the seedling. There will always be a small portion of the endosperm remaining on the seedling, but it is better to leave a small piece than to severely damage the seedling. You should have extra seeds when you get to this step, in case some endosperms are not successfully removed. You will need 6 seedlings for each bottle in this experiment.

1. Prepare squares of foil (approx. 15 x 15 cm) before starting the operation, label the foil with a marker, indicating the name of the experiment, and the replicate number. Weigh the pieces of foil, record the weight on both the foil and on the worksheet.
2. Spread filter paper or plain paper on a desk a and prepare seedlings of maize.
3. Cut off the endosperm carefully with the razor blade or scalpel (See Figure 1).

Attention!!! Do not throw away endosperms that have been removed from seeds!

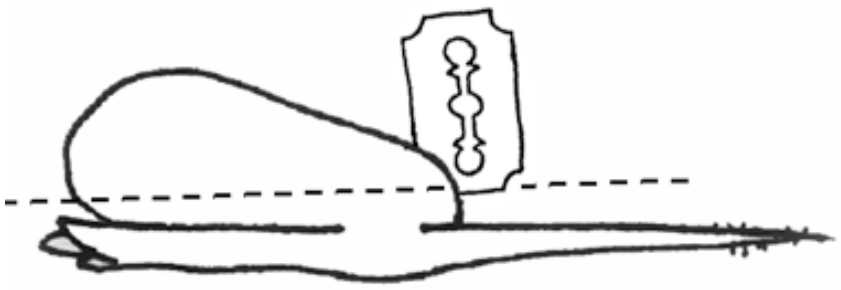
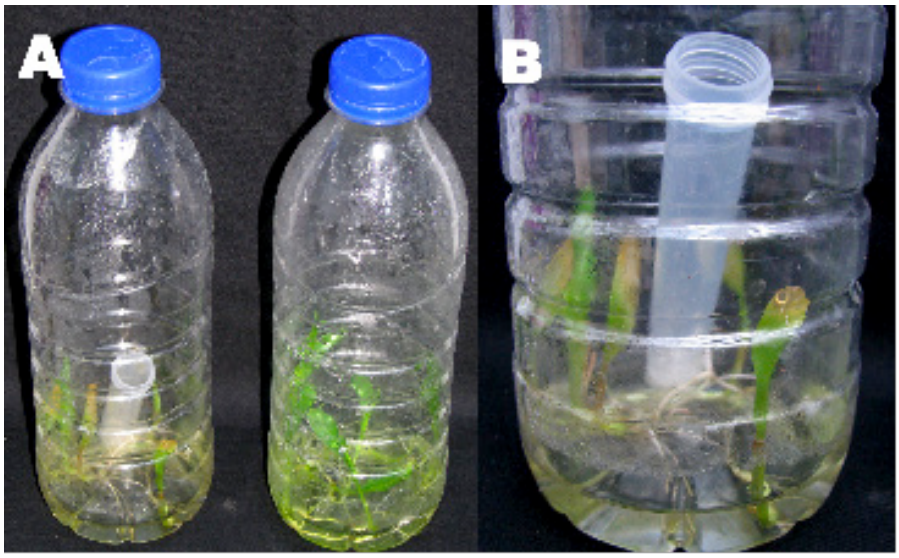


Figure 1: Direction of incision through the seedling during removal of endosperm.

4. Place the removed endosperms onto the labeled foil squares. Each foil square will be folded into a packet containing the 6 endosperms from the 6 seeds assigned to one bottle. Be sure to leave the label visible on the folded foil packet.
5. Make several punctures with scissiors thoroughly through the foil, which wiill allow for the evaporation of water.
6. Put the foil packages into the oven and let dry them at 90 °C for 8-12 hours (until the next day).
7. Weigh the dry endosperm packets – you don´t have to remove the endosperms from the packet, you will weigh the whole foil packet and record this weight in the protocol – this value will be used in determinating increase of biomass in final stage of the experiment.

Plant and Observe Seedlings

1. Transplant 5-6 young seedlings from the germination tray into each bottle.
2. Weigh out 2 g of sodium hydroxide (NaOH) and add to the test-tube or small bottle. ***ATTENTION! Sodium hydroxide is a strong base – DO NOT touch it with your fingers.***
3. Place the test-tube containing sodium hydroxide carefully with tweezers into the bottom of the bottle. Ensure that the test-tube remains vertical to prevent the fertilizer solution or plants from coming into contact with the sodium hydroxide.
4. Close the bottles carefully and don´t open them during the experient.



5. Place bottles in a well lit area, but avoid direct sunlight to reduce potential for overheating.
6. Grow plants at room temperature for 14 days. Observe plants daily and record any differences between the CO_2 treatments (Table 1 of the *Laboratory Data Sheet*). Begin observations on day of transplant.

Journal Question: What differences have you noticed after 7 days of cultivation? Do your observations agree with your original hypothesis? Explain.

Harvest the Plants and Evaluate Biomass

Materials and Tools (*per replicate*)

- ❑ Sink / washbasin with tap water
- ❑ Plastic trays (it is possible to re-use the germination trays)
- ❑ Scissors (ideally fine surgical ones or nail scissors) or razor blade
- ❑ Aluminium foil
- ❑ Permanent marker
- ❑ Pencil
- ❑ Laboratory scale (accuracy of 0.01 gram)
- ❑ Absorbent paper (paper towels, filter paper, etc)
- ❑ *Laboratory Data Sheet* - Carbon Dioxide
- ❑ *Data Summary and Analysis Sheet*

  **Note: kiln or drying oven is also necessary

Harvest Procedure

All plants from each bottle will be harvested together.

1. Before harvesting plants prepare 2 squares of aluminium foil (approx. 15 x 15 cm each) for each bottle: one for roots and shoots. Label them with a marker – write the treatment information, such as **roots, decreased CO_2** and number of replicate.

2. Remove the tape, the top of the bottle and then the plants. Plant roots may be interlaced; therefore harvest and weigh all the plants from each bottle together (1 group of roots and 1 group of shoots).

3. Use scissors to cut the shoots from the roots. Place the roots in a plastic tray containing tap water to prevent drying.

4. Package shoots into the labeled foil squares – KEEP LABELS VISIBLE. Repeat the process for roots.

5. Puncture the foil envelopes/packets several times using the small point of the scissors, a pin or a paperclip to allow evaporating water to escape.

6. Weigh all foil packets and record the fresh weight on Table 2.

7. Place the packets into kiln or oven at 90 °C and dry them for 8 to 12 hours. It is also possible dry them at lower temperatures but for a longer time (e.g. 60 °C for 2 to 3 days).

Report Results

1. Remove the foil packets and weigh individually on the scale. Record your packet dry weight value on your *Laboratory Data Sheet* (Table 3).

2. Follow instructions in the harvest section of the *Laboratory Data Sheet* to calculate:
   a. Increase in biomass (in grams of dry weight) (Table 4)
   b. The root-shoot ratio using plant dry weights (Table 5)
   c. Compare results between experimental treatments in the *Data Summary and Analysis Sheet* Table 6.
3. Graph interesting and/or important results.

Conclusions

1. Revise answers to questions posed at the beginning of the experiment in your science notebook or on *Student Laboratory Questions* sheet. Does the experimental outcome provide the answers or at least a clue?

2. Evaluate validity of your hypotheses. Were they supported or rejected? What was your evidence?

3. Did you encounter any issues/difficulties while performing the experiment? What were potential sources of error in the experiment? Are there ways the procedure could be improved?

4. Record any remaining questions about the experiment or its outcomes. How would you design an experiment to test one of these questions?

5. All scientists, once they have completed their investigation, share their findings with peers in their community. Follow the instructions provided by your teacher to share your work

Plant-A-Plant Student Worksheet – Carbon Dioxide

Students: _____ Replicate no. _____

OBSERVATIONS AND CALCULATIONS (per replicate)

Record data observations and calculations in tables one through five. Shaded cells indicate a calculation is necessary (required equations included below). Tables are designed for a single replicate. Photocopy these tables (pages 6-9) in order to record data for all of your replicates (e.g., bottles per treatment).

Plant and Observe Seedlings

During cultivation, besides measuring and recording the temperature, you may notice differences between experimental treatments. Plant height or changes in shoot leaf color may be some of the notable differences observed. Use a ruler to estimate average seedling height for each flowerpot. Record your observations in Table 1.

Table 1: Observations of Plant Characteristics (dependent variables)

Day of Cultivation	Height Comparison		Shoot Color Changes		Additional Observations or Questions (use backside of data sheet if necessary)
	Decreased CO_2	Control	Decreased CO_2	Control	
1					
2					
3					
4					
5					
6					

Table 1: Observations of Plant Characteristics (dependent variables) Con't

Journal Question: What differences have you noticed after 6 days of cultivation? Do your observations agree with your original hypothesis? Explain.

Day	Height Comparison		Shoot Color Changes		Additional Observations or Questions (use backside of data sheet if necessary)
	Decreased CO_2	Control	Decreased CO_2	Control	
7					
8					
9					
10					
11					
12					
13					
14					

Harvest Plants and Evaluate Biomass

All plants from each flowerpot should be treated as a set and harvested together.

Whole Plant = shoot + root. Seed is not included.

Mark the foil with replicate number and treatment type.

Table 2: Fresh weight of whole plants and parts			
Treatment (independent variables)	Fresh weight in foil (g)		
	Shoots	Roots	Whole Plant
Decreased CO_2			
Control			

Table 3: Dry weight of whole plants and parts (dependent variable)			
Treatment (independent variables)	Dry weight of plant parts in foil (g)		
	Shoots	Roots	Whole Plant
Decreased CO_2			
Control			

Don't weight it - calculate it!

Calculations

Weight whole plant = Weight shoot + Weight root

Table 4: Increase in biomass (dependent variable)					
Treatment (independent variables)	Average fresh weight of seed (g) **	Dry weight of seed (g)	Dry weight of seed group (g)	Dry weight of whole plant	Increase in biomass (dry matter in g)
Decreased CO_2					
Control					

Important notes for calculating increase in plant biomass.

Plants consist mainly of water. Water content in leaves is about 60-90%. In contrast, seeds contain only 12% water.

When calculating the increase in maize biomass, you need to know the initial dry weight of the seedlings you have used for planting. However, because it is impossible to measure the dry weight of a seed without damaging it and preventing its ability to grow, we must use the assumption above that seeds contain 12% water. Therefore 88% of the seed's mass is its dry weight.

Remember, you are working with an entire set of plants from a watering system tray; therefore you must multiply the average weight with the appropriate number of plants.

Example: Initial average weight of a seed was 0.420 g, dry matter is 88%.
Average dry weight of seed= 0.88 x 0.420 g = 0.370 g.
You have 10 seeds in one experimental system, thus:
The average dry weight of the seeds = 10 x 0.370 g = 3.7 g.
Increase in biomass = Dry weight of harvested plants - 3.7 g.

Calculations:

** = from germination datasheet
Dry weight of seed = Average fresh weight of seed** x 0.88
Dry weight of seed group = Dry weight of 1 seed x Number of plants in the treatment (tray)
Increase in biomass = Dry weight of whole plants – Dry weight of seed group

Table 5: Weight Ratio- root:shoot (dependent variable)			
Treatment (independent variables)	Dry weight of roots (g)	Dry weight of shoots (g)	Ratio root:shoot
Decreased CO_2			
Control			

The root:shoot ratio is one measure to help you assess the overall health of plants. The root:shoot ratio measures the allocation of carbon in the form of photosynthate to the roots (below ground tissue) and shoots (above ground tissue). Environmental stimuli (e.g., light, CO_2) may influence carbon.

Calculations:

Root:shoot ratio = Dry weight of roots / Dry weight of shoot

Plant-a-Plant Student Laboratory Questions: **Answer the questions and identify the sources for your ideas (D,B,S).**

	Pre-Lab: Existing Knowledge	Can this question be answered by this experiment? (Y/N)	**Post-Lab: Knowledge Gained**	To what extent was the question answered? (All, In, part, Not at all)
How does a lower concentration of carbon dioxide (CO_2) in the air affect plant growth?				
Is CO_2 a limiting factor to growth?				
How does CO_2 from the air get into plant leaves?				
How is CO_2 stored in plants?				
How does increased CO_2 in the air affect plant growth and carbon storage?				

Identifying Sources for Ideas (adapted from GLOBE Earth Systems - LC1)

For each laboratory question identify what kind of source you used for the idea. Record the designation by writing the letter next to your idea and circling it for distinction.

D – Your answer is based on **data**. Use "D" to designate an idea for which you have collected or seen supporting data. Data could have been collected by your class, another GLOBE school, or others.

B – Your source is **background information**. Use "B" to designate an idea that you have recalled from a previous reading or experience in another course, at home, or elsewhere, and that you could actually find and bring to class. There may be data somewhere to substantiate this information, but you have either not seen it or do not have access to it.

S – Your source is **speculation**. Use "S" to designate an idea based on scientifically informed speculation. This is your opinion founded on what you have learned over time, but you can not point to a particular source of data or other information to support it. (Creative speculation – when based on authoritative background information and data – is one of the keys to excellent scientific work.)