Plant-A-Plant Carbon Dioxide II Laboratory Guide

Task

Plant maize seedlings in a semi-closed system (plastic soda bottle within a jar). Observe the effect of elevated carbon dioxide concentration on the growth of plants. Harvest plants and compare the characteristics between treatments.

Prepare and Perform the Experiment

Materials and Tools (*per replicate*)

- ❑ 20 Maize seedlings
- ❑ Four transparent plastic 2-liter bottles
- ❑ Four 800 ml jars/cans (diameter of jar must be large enough to fit the plastic bottle inside)
- ❑ Four small pots or cups (approximately 5 cm tall, must fit inside plastic bottle)
- ❑ Fertilizer containing basic nutritients (for example, Kristalon Start or Miracle Gro)
- ❑ Distilled water
- ❑ Carbonated water
- ❑ Sand
- ❑ Candle or burner
- ❑ Measuring cylinder
- ❑ Pencil and permanent marker
- ❑ Masking tape for labeling
- ❑ Sharp scissors
- ❑ Razor blade or scalpel
- ❑ Needle

** Note: At least <u>two</u> 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 thouroughly - 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. Measure 9-10 cm from the bottom of the bottle and cut the bottle into two parts.

4. Carefully make 8-10 holes with the sharp scissors into the bottle bottom. These pores will serve for carbon dixide transport from the soda solution to the plants.

5. Light the candle or burner, heat the needle and use it pierce a hole into the upper part of the bottle. This hole is needed for pressure equalization inside the growth chamber.

6. Assemble the experimental system to determine amount of water needed.
   a. Turn the bottom part of the bottle (perforated with 8-10 holes) upside down and place the upper part of the bottle on top of it to create the *growth chamber*.
   b. Place the growth chamber inside 800 ml jar.
   c. Fill the 800 ml jar with water so that the joint of bottom and upper part of the growth chamber is submerged, but the upper part of the growth chamber including the

cup with plants is not (See Figure 2 in the Appendix).

7. Temporarily dismatle the experimental system. Measure the volume of the water in the jar and record it on your data sheet.

8. 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 experiment.

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 5 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 your data sheet.

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!

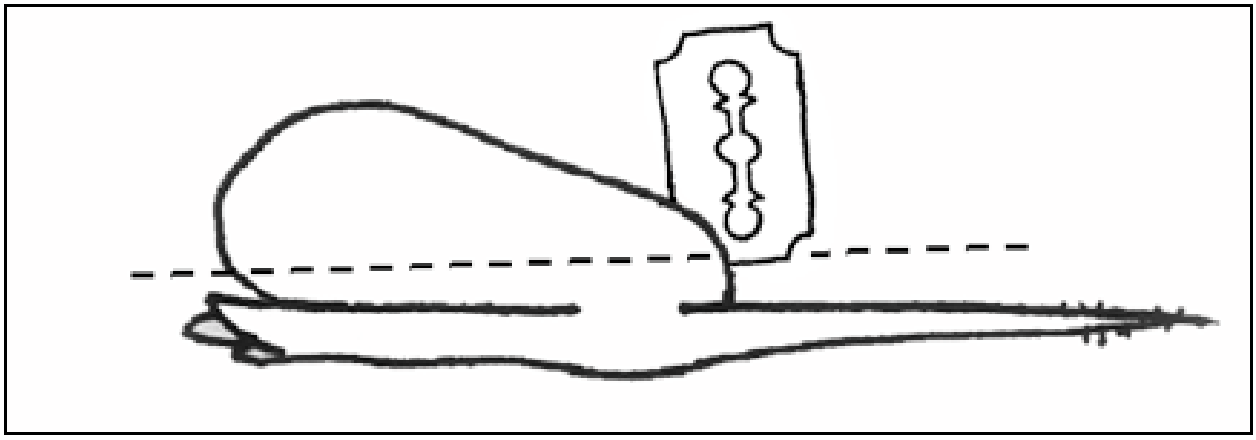

Figure 1. Direction of incision through the seedling during removal of endosperm.

4. Place the removed endosperms onto the labeled foil squares. Each foil square will be folded into a packet containing the 5 endosperms from the 5 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 on your data sheet – this value will be used in determinating increase of biomass in final stage of the experiment.

Plant and Observe Seedlings

1. Carefully place the maize seedlings with the endosperms removed into the sand. Put 5 seedlings into each cup and irrigate with 30 ml of the fertilizer solution.
2. Place the cups wtih plants into the upper part of each growing chamber.
3. Place the growth chambers into the 800 ml jars and using Table 1, measure and pour in the correct amount of tap vs. carbonated water. The exact amount will depend on the volume of water you measured in Step 7 of the Preparation section. **Note:** Work quickly with the carbonated water to prevent CO_2 losses.
4. Label each system.

Table 1. Tap vs. Carbonated Water ratio for each treatment

Treatment	Carbonated Water	Tap Water
Control	0	Entire Volume
CO_2 -1	1 part	2 part
CO_2 - 2	1 part	1 part
CO_2 - 3	Entire Volume	0

5. Place bottles in a well lit area, but avoid direct sunlight to reduce potential for overheating.
6. Change the CO_2 enriched solutions every other day. Grow the plants for 12 days, or until the plants have run out of space. Observe and record any differences between treatments.

Harvesting Plants and Evaluate Biomass

Materials and Tools (*per replicate*)

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

** Note: kiln or drying oven is also necessary

Harvest Procedure

You will harvest all plants from one bottle together in one group.

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, CO_2 -1*** and number of replicate.

2. Open the growth chamber and carefully remove the cup with plants. Remove plants from the substrate being careful not to break the roots and place them in a plastic tray filled with tap water. Wash roots completely, do not leave grains of substrate on them (especially important for sand). Keep plants in water until they are ready to be weighed. Plant roots may be interlaced, so you will harvest and weigh all the plants in one 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 your data sheet.

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 data sheet

2. Calculate and record important values such as:
   a. Fresh weight of entire plant (weight root + weight shoot)
   b. Dry weight of entire plant (weight root + weight shoot)
   c. Water content in fresh plant biomass (%) for entire plant and for individual parts
   d. Increase in biomass (grams of dry weight, in %)
   e. Dry weight ratio of roots:shoot

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.

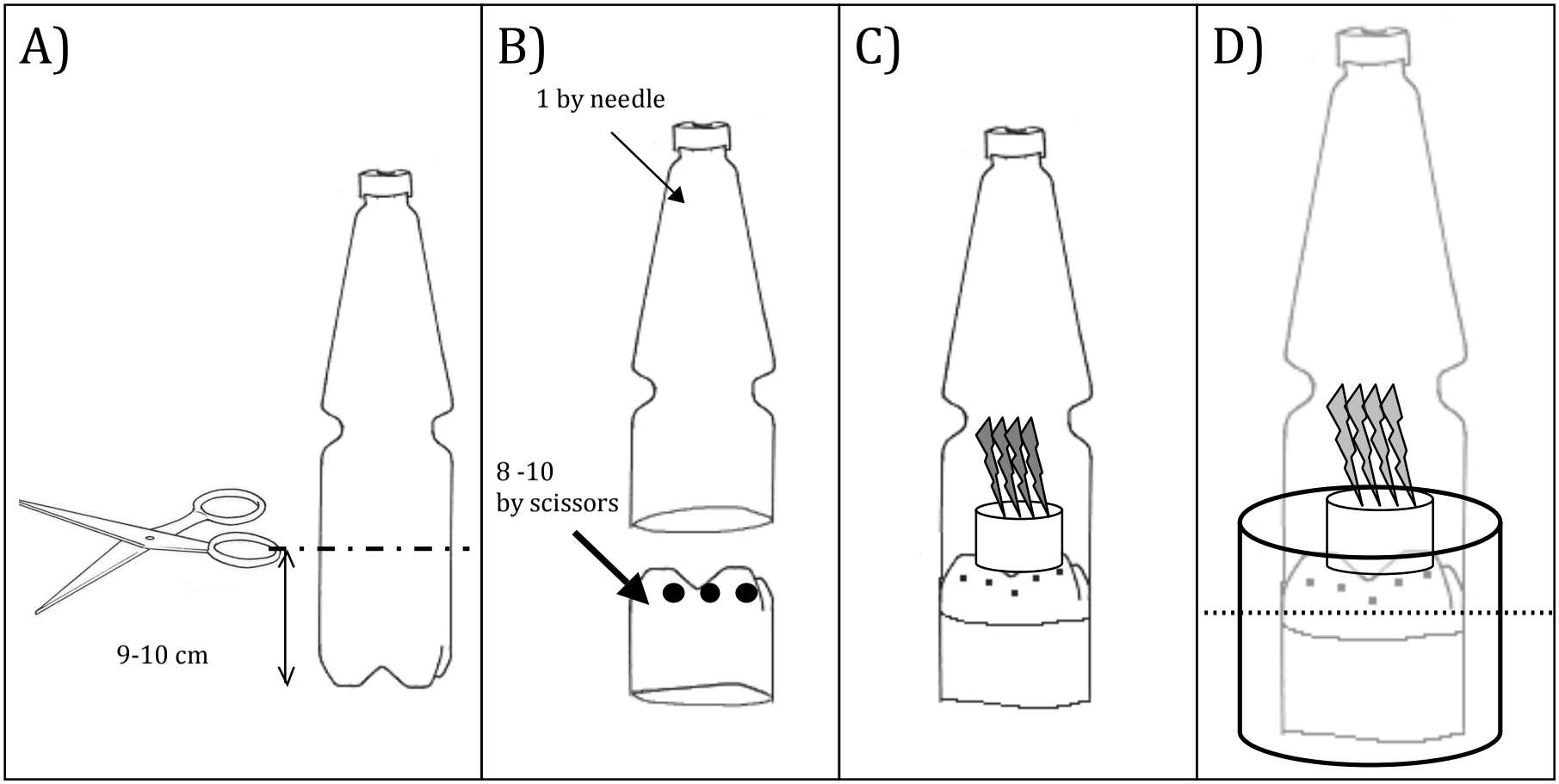

Figure 2: Setting of the Experimental system: A) Cut the plastic bottle 9-10 cm above the bottom. B) Position of the pores – one small pore pierced by a needle on the upper part, 8-10 bigger pores by scissors on the bottom part. C) Place the cup with barley in the growth chamber. D) Place the growth chambre into the jar with carbonated / tap water solution. IMPORTANT: dotted line (2D) indicates the solution level – **The joine of the two parts of the growth chamber must be submerged, but pores for CO_2 diffusion must be above the solution level!**