Name ______________________ Class ______________ Date ____________

Guided Research Lab 2

Cellular Respiration in Fast Plants

Part I: Procedure

Materials

- goggles
- hot-glue gun
- 3–4 one-quarter inch flat metal washers
- permanent glass-marking pen
- thin-stem dropping pipettes
- absorbent cotton
- nonabsorbent cotton
- germinating Fast Plant® seeds (or other material or organism)
- glass beads (or dry, baked Fast Plant seeds)
- Celsius thermometer
- centimeter ruler
- manometer fluid (soapy water with red food coloring)
- constant-temperature water baths
- 2 1-mL tuberculin syringes without needles
- 40-μl plastic, capillary tubes
- 15% solution of KOH, potassium hydroxide solution

Part A: Constructing a Microrespirometer

1. Plug in the hot-glue gun and allow it to heat up.

2. Take a tuberculin syringe (without a needle) and make sure that its plunger is pushed all the way in.

3. Carefully insert a 40-μl plastic capillary into the syringe where the needle normally would be. Insert it as far as the plunger tip but no further. This will help to prevent the capillary from becoming plugged with glue.

4. While holding the capillary tube straight up, add a small amount of hot glue around its base (where it meets the syringe). This seals the capillary to the syringe. Keep the capillary pointed straight up until the glue cools—this should not take long. If needed, add a bit more glue to ensure an airtight seal between the capillary and the syringe.

5. After the glue has cooled, pull back on the plunger and make sure that the glue has not plugged the capillary. If the capillary is plugged, carefully remove the glue and the capillary and start over.

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Part B: Preparing the Microrespirometer

1. Draw a small quantity of manometer fluid into the full length of the microrespirometer's capillary tube. Then eject the fluid back out of the capillary. This coats the inside of the tube with a thin soapy film that helps prevent the manometer fluid from sticking.
2. Carefully insert a small plug of absorbent cotton into the barrel of the microrespirometer, all the way into the "0" mL or cc mark. You can pack the cotton to the end with the barrel of a clean thin-stem pipette.
3. Add 1 small drop of 15% KOH to the cotton in the microrespirometer. Be careful to no add too much. **CAUTION:** Make sure you protect your eyes with goggles because KOH is caustic.
4. Add a small plug of nonabsorbant cotton on top of the absorbent cotton plug already inside the barrel of the microrespirometer. You can pack the cotton to the end with the barrel of a clean thin-stem pipette. This nonabsorbant plug is needed to protect the seeds from the caustic KOH.
5. Slowly reinsert the syringe plunger. **CAUTION:** Be careful to point the capillary tip into a sink or container. There may be excess KOH in the syringe that might squirt from the end of the capillary. Push the plunger in until it reaches the cotton so that any excess KOH is removed. Remove the plunger to add seeds.
6. Add the 0.5 mL germinating seeds to the microrespirometer. Push the plunger in to the "1.0 ml" mark. This creates a sealed microrespirometer chamber with a 1-milliliter volume.
7. Place 3–4 washers around the barrel of the microrespirometer. The washers provide weight so that the microrespirometer will sink.
8. Place the microrespirometer assembly in a room temperature (about 20°C) water bath. Adjust the level of the water bath so the capillary tube is sticking out of the water while the barrel of the microrespirometer is completely submerged. In order to easily read the capillary tube, it must be out of the water. Make sure the capillary tube is open (not sealed).
9. Repeat steps 1–8 for preparing additional experimental microrespirometers in warm (about 40°C) or cold (about 0°C) water baths.

Part C: Setting Up Your Control (optional)

Because a microrespirometer is so sensitive to changes in gas volume, it is also extremely sensitive to changes in temperature and air pressure. Thus you will use a control microrespirometer to measure and correct for temperature and pressure changes that occur while using the microrespirometer. The thermobarometer (control) is set up just like the microrespirometer except that it contains nonliving matter instead of germinating seeds.

1. Add the 0.5 mL of beads or baked seeds to the second microrespirometer you made in Part A. You do not need the KOH for the control. Reinsert the syringe plunger and push it in to the "1 mL" mark. This seals the chamber and creates a chamber that has the same volume as the experimental microrespirometer.

2. Place 3–4 washers around the barrel of the control.
3. Place the assembled control in the water bath next to the experimental microrespirometer. Adjust the level of the water bath so the capillary tube is sticking out of the water while the barrel of the control is completely submerged. In order to easily read the capillary tube, it must be out of the water. Make sure the capillary tube is open (not sealed).

Part D: Collecting Data

1. Print a spreadsheet template of Table 1 to record your data and observations. Use a separate data table for each microrespirometer. Place the thermometer into the water bath along with the microrespirometer and the control. Wait 5 minutes to allow the temperature in the microrespirometers to equalize.
2. Use a dropping pipette to add one small drop of manometer fluid to the tip of each capillary tube. If everything is working properly, the drop will be sucked down into the capillary tube. The manometer fluid will seal the chamber of the microrespirometers. (You should use the plunger on the control microrespirometer to get the manometer fluid into the capillary. Pull on the plunger until the manometer drop is about half-way down the capillary.)
3. As oxygen is consumed, the manometer fluid plug will move toward the chamber. Record the starting position of each plug by marking its position on the capillary with a marker. Be sure to mark the bottom edge of the plug. These are your Time 0 marks. Begin timing once you have made the Time 0 marks.
4. At five-minute intervals, measure the temperature of each water bath and mark the position of the manometer fluid for each capillary tube. Be sure to mark the bottom edge of the fluid plug. Continue marking the positions until the fluid in the microrespirometers has traveled the entire length of the capillary, or until 25 minutes has passed. Record the temperature reading in Table 1.
5. Remove the microrespirometers from the water baths. Use a centimeter ruler to measure the distance from the initial mark (Time 0 mark) to each of the five-minute intervals marked on each capillary tube. Record your measurements for each microrespirometer in column C of the appropriate data table.
6. To calculate the change in fluid position during each time interval (column D values), subtract the fluid position (column C value) at the beginning of the time interval from the fluid position (column C value) at the end of the time interval.
7. Repeat for the second microrespirometer.

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TABLE 1 : Results

A Total time (minutes)	B Water bath temperature (°C)	Microrespirometer C Total distance fluid has moved (cm)	D Change in fluid position during time interval (cm)
0			
5			
10			
15			
20			
25			

Analysis

1. Graph the total distance the fluid has moved (column C) versus the total time data (column A). Time is the independent variable and is plotted on the x-axis, while the oxygen consumed is the dependent variable and is plotted on the y-axis. Graph both temperature treatments on the same graph.

2. The slope of the best-fit line represents the rate of oxygen consumption, or rate of respiration. Calculate the rate of respiration for the germinating Wisconsin Fast Plant seeds at each temperature. How does temperature affect respiration in seeds? How would you improve the design of this experiment?

Conclusions

The main purpose of this investigation is to develop the skill of using microrespirometers so you can design your own investigation of cellular respiration. The experiment you've just completed only begins to answer the questions of the effect of temperature on seed respiration. There is much more that can be done.

Now that you have completed the investigation, you probably have a few questions about cellular respiration in germinating seeds. Enter two questions into your lab notebook (about germinating seeds and cellular respiration) that you could investigate further. These questions will serve as a starting point for developing your own investigation in the next section. Before you write your questions you may want to review the lab techniques you used and the results that you obtained.

1. What are probable sources of error in the lab?

2. What factors might alter the rate of cellular respiration?

3. Can you develop a method to test for a particular factor?

Your questions might focus on the seeds themselves, the environmental conditions, or something else altogether—it's up to you!