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Construction of a Phloem Pressure-Flow Model

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Students build artificial cells that model phloem sap transport. Students observe a working two-cell pressure-flow model and then build their own model from materials available in the laboratory. Students use trial and error to get their apparatus to work well enough to cause overflow of one of the beakers. Discussion questions guide students in their understanding of pressure-flow as they build the model. Students gain confidence in the construction of customized equipment and in their understanding of source-to-sink flow of carbohydrate-rich phloem sap.

Keywords: Pressure-flow hypothesis, phloem, plant physiology, osmosis.

Introduction

Students are not often asked to *build* something. Many students find this construction activity a welcome break from cookbook labs and from guided inquiry labs, each of which requires a different kind of attention and effort.

Understanding the transport of phloem sap requires a working appreciation of osmosis and water potential. In preparation, I conduct an "osmosis clinic" in lecture where I draw a series of scenarios on the board that demonstrate the net flow of water across a selectively permeable membrane in a beaker. These drawings are intentionally similar to the three-dimensional working models the students will soon build. Students have already demonstrated aspects of cohesion tension theory in the laboratory, and they are familiar with the solute and pressure aspects of water potential. I also use typical textbook figures to teach the Münch pressure-flow hypothesis in the lecture before this scheduled laboratory activity.

When the lab begins, a phloem sap transport model made of plastic bottles, dialysis tubing, plastic or glass tubing, beakers, and dyed corn syrup is running at the front of the room. I identify the materials used in the model, the "source cell" and the "sink cell." I offer minimal explanation of osmosis and pressure-flow at this time, although both have already been discussed in lecture. The assignment is for students to build their own working model from the same materials and to discuss the assigned discussion questions as they labor.

Formal assessment for the laboratory activity is based entirely on getting the model to work. Although the students begin the activity within the class period, the test of their model (overflow) is likely to happen long after they have left the lab. If the model fails, they can try again until it does finally work. There is no time limit on this assignment, and there is no writing assignment. However, students are assessed on the concepts of osmosis and pressure-flow in an ordinary lecture exam.

This activity requires careful observation of the running model. Students begin to question what elements of construction are important for the model to work. Through trial and error, for example, tight seals will become important. Many students also gain a first-hand knowledge of the limitations of the model and the materials. They might ask how is dialysis tubing really like a cell membrane? Why does the plastic bottle have "windows?" What part of the model represents xylem?

This activity is designed for most of a 170 minute laboratory class with 20-24 students. Students generally work in pairs. My introduction to the working model takes about 20 minutes. The most efficient students have their model built by 70 minutes into the period. It takes another 10 minutes to verify that the model is working (colored liquid is being pushed out of cell A). Slower workers and students who need to revise their set-up usually have their system running by 130 minutes into the period. This activity has been implemented with upper-division biology majors in a plant physiology class at a small liberal arts college. A modified version of this laboratory activity has also been implemented in a large scale non-majors general botany class at a public institution.

Student Outline

Introduction

The pressure-flow hypothesis is to phloem transport what cohesion tension theory is to xylem transport. Whereas as xylem sap moves by negative pressure, phloem sap moves by positive pressure generated by a strong osmotic gradient at source cells. The pressure-flow hypothesis is credited to Ernst Münch (1930) but the concepts underlying the hypothesis much precede this oft cited monograph (Knoblauch and Peters, 2010). Münch's name is associated with pressure-flow because he so effectively demonstrated the plausibility of the hypothesis with a working model that he set up at the German Botanic Society meetings of 1926 and 1927 (Münch, 1927). Interestingly, this hypothesis lacks definitive support because of technical difficulties in measuring the pressure potential and osmotic potential of phloem sap (Knoblauch and Peters, 2010 & 2013). It is only recently, during the summer of 2014, that Michael Knoblauch of Washington State University began to gather data that will likely either support or undermine the Münch pressure flow hypothesis (Fountain, 2014). In order to carry out the necessary measurements, Knoblauch had to develop sophisticated pressure gauges to penetrate live sieve elements. His results are expected to be published some time in 2015.

Today you are going to build an Ernst Münch pressure-flow model, and perhaps you will be convinced of its elegance in demonstrating that the pressure-flow hypothesis is plausible. The instructor has set up such a model: it is an apparatus that simulates two plant cells connected by phloem tissue. The two connected cells are a model for the phloem symplast. Artificial sieve tube element A (the source) initially contained a mixture of corn syrup and food dye. The corn syrup represents phloem sap while the dye helps us visualize its movement. Artificial sieve tube element B (the sink) initially contained only water. Between the source cell and the sink cell is a hollow tube representing the sieve tube between cell A and cell B. Each artificial cell has a hard plastic housing that simulates a cell wall. The cell membrane is simulated by dialysis tubing (a semi-permeable membrane) threaded inside the housing and sealed by the plastic screw top lid at the top end (see Figure 1) and by a tight string knot at the bottom end. Both beakers initially contain the same amount of water. I suggest that you mark the water level in each beaker so you can monitor the progress of your set-up.

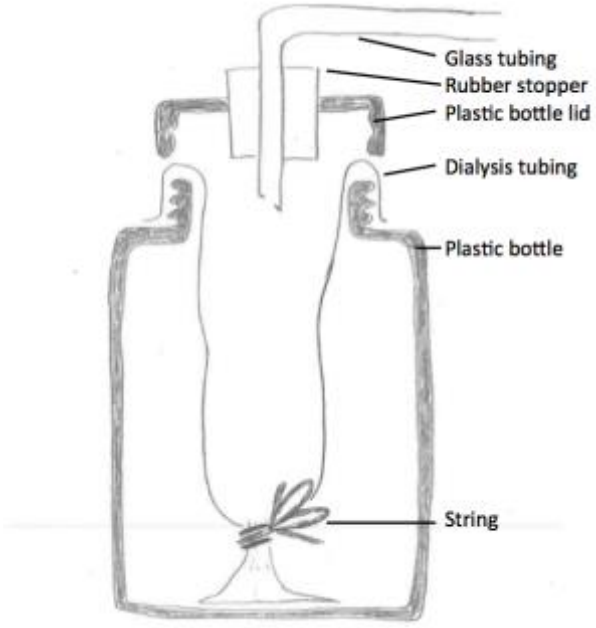

Figure 1. Set-up of one model cell. The dialysis tubing is secured at the top by the screw top bottle lid and at the bottom is secured with string. A rubber stopper with glass tubing penetrates the plastic bottle lid.

Assignment

Scientific process often includes design and construction of customized equipment and models, but biology courses rarely make demands on students to make their own models. Here, you will practice mimicking the construction of a phloem transport model. Through hands-on construction of the model, you will be able to visualize and better-understand the concepts behind the pressure-flow hypothesis for phloem sap transport.

Assemble a *working* phloem transport model like the one operating in class. You will be provided with the appropriate raw materials and tools. When you have finished assembly of your cells, you should start running the model with

the water level 1 or 2 cm below the rim of the beakers. Your assignment credit is dependent entirely on your set-up functioning well enough for the water level in beaker B to overflow without any outside intervention. Overflow may occur long after class is over, so you need to provide secondary containment for your set-up.

Questions

What physiological mechanisms explain the movement of water and corn syrup in this system? Answer the following questions based on what you observe and infer from the working model. It will help you to discuss these questions with your classmates and instructor while the model is running in front of you.

1. When this apparatus was initially set up, which had the lower water potential—the water in Beaker A or the phloem sap (corn syrup) in Cell A?
2. Was there initially a water potential difference between the contents of Beaker B and Cell B?
3. Now that the system at the front of the room has been running for a while, what has changed from the initial conditions?
4. What exactly is happening at the membrane separating the liquid in Beaker A and the liquid inside Cell A?
5. What evidence is there of fluid moving through the model sieve tube? Explain.
6. Is there now a water potential difference between the water in Beaker B and the contents of Artificial Cell B (see question #2)? What is the direction of net water movement? Which chamber has the higher water potential? Why?
7. After the model has run for about an hour, what has happened to the water level in Beaker A? What has happened to the water level in Beaker B?
8. Although our artificial phloem transport system runs spontaneously, without any added energy, it will not continue to run indefinitely. In contrast, what allows the sieve tubes of a living plant to continue to function indefinitely?

Materials

The following materials are required for each group:

- The sieve tube
  - Two rubber stoppers. Each stopper needs a hole drilled into it that matches the size of the plastic/glass tubing. Your local chemistry department probably has a drawer full of these already drilled out.
  - 1 mL glass pipette bent at right angles approximately 5 cm from each end to form an inverted "U." Glass pipettes should have stoppers already attached when presented to students (so they don't cut themselves jamming stoppers onto glass). Alternatively, approximately 1 foot of plastic tubing to connect holes in rubber stoppers.
- The cell wall
  - Two wide mouth screw cap plastic bottles such as 8 oz Nalgene polyethylene bottles from REI (item # 4020540003). The plastic bottle must be soft enough to cut but hard enough to deal with the strain of being handled.
  - Two plastic bottle caps with a hole drilled in the middle of each cap. The hole must match the size of the rubber stoppers.
  - Flexible plastic ruler.
  - Blunt scissors for cutting the plastic bottles.
- Phloem sap
  - Corn syrup, 150 mL.
  - Stirring rod and jar to hold very sticky used stirring rod.
  - Food coloring: red, blue and green. Groups use about 3 drops per 150 mL aliquot of corn syrup.
  - A jar or disposable plastic cup in which to mix the corn syrup and food coloring.
  - A funnel that matches the size of the hole in the plastic bottle lid.
- The cell membrane
  - Dialysis tubing, two strips each about 10 cm long, but must be custom cut to the length of the artificial cell plus about 2 cm to fold over the threads of the bottle. Tubing should be 3 inches wide when pressed flat. Narrower dialysis tubing is okay if it fits around the threads of the plastic bottles you are using. The match up between the plastic bottle opening and the dialysis tubing is crucial, and should be worked out first.
  - Simple string to tie off the dialysis tubing at the bottom.
- The apoplast
  - Two 600 mL beakers. Larger sizes, up to 1 L are okay. Regardless, the students need to match their cell size to the beakers they have.
  - Tap water.
  - Secondary containment (flat bottom and at least 12 x 6 inches, 1-2 inches high). Ideally this container is strong enough so that you can move the whole model if needed.

Notes for the Instructor

Students will be cutting "windows" into their plastic bottles in order to simulate the easy flow of water through the cell wall. The windows also allow easy viewing of the dyed corn syrup into Cell B.

It is helpful to encourage thorough mixing of the dye with the corn syrup. Some students are slow to realize how important even mixing is for pressure flow visualization.

This activity can be modified with more or fewer steps for the students to complete themselves. Jamming glass pipettes into rubber stoppers is inherently dangerous, so this can be done ahead of time, or plastic tubing can be used instead of glass. The tradeoff is that the glass pipettes run more smoothly than the flexible plastic tubing. With the plastic tubing, the corn syrup sometimes enters the sink cell in spurts instead of in a continuous flow.

The only safety concern besides broken glass is the use of scissors. Cleaning-up corn syrup can be tedious because it is so viscous and sticky. Warn students about thoroughly cleaning up the inevitable spills.

Sample Results

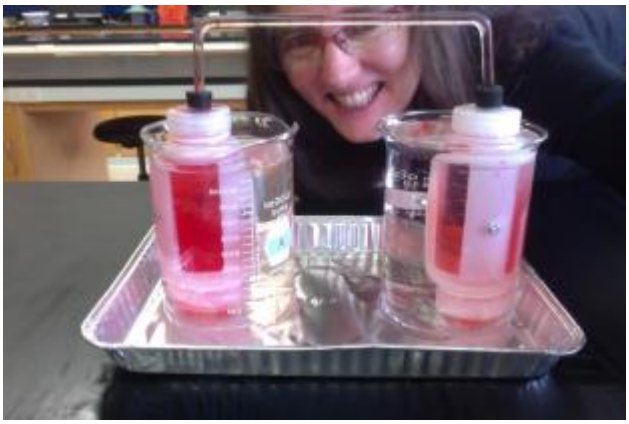

Figure 2. A pressure flow model built by students at Whitman College. The source cell (Cell A) is on the left. Note the water level in the two beakers. Pressure from the source cell has already caused the right beaker, with Artificial Cell B, to overflow.

Acknowledgements

Many thanks to Matt Ritter for introducing me to this phloem transport model. This activity is heavily modified from Ritter et al (2012). Thanks to Allison Calhoun for help with bending the glass pipettes and to Larry North for help with drilling out the plastic lids.

Literature Cited

Fountain, Henry. 2014. Exploring a tree one cell at a time. *New York Times*. September 1, 2014.

Knoblauch, M., and W.S. Peters. 2010. Münch, morphology, microfluidics—our structural problem with the phloem. *Plant, Cell, & Environment*, 33:1439–1452.

Koblauch, M. and W.S. Peters. 2013. Long distance translocation of photosynthates: a primer. *Photosynthesis Research*, 117:189-196.

Münch, E. 1927. Versuche über den Saftkreislauf. *Berichte der Deutschen Botanischen Gesellschaft*, 45**:** 340–356.

Münch, E. 1930. *Die Stoffbewegungen in der Pflanze*. Gustav Fischer, Jena, Germany.

Ritter, Matt, David Keil, and Susanne Altermann. 2012. *General botany lab manual*. California Polytechnic State University San Luis Obispo, 96 pages.

About the Author

Susanne Altermann earned her Ph.D. in ecology and evolutionary biology from University of California Santa Cruz. She currently teaches courses on diversity of life, ecology, symbiosis, and plant physiology at Whitman College in Walla Walla, Washington. Her research interest is the geographic structure of symbiotic partnerships.

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