The Drip Races
A hands-on activity to teach plant water transport

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Concept and purpose

The purpose of The Drip Races is to teach plant water transport concepts using a simplified version of a device called a hydraulics manifold, which is used in real scientific studies to measure the rate of water transport in plants. This document details the lab procedure, scientific and information resources, and the Drip Races power point provides image references for the concepts presented in this document. We provide pertinent information the properties of the plant vascular system, how those properties translate into plant growth and drought survival, and how plant water transport properties differ across species depending on their leaf traits and the environmental conditions where they live.

The Drip Races can be set up as a demonstration, a hands-on activity, or a fully-fledged experiment. The device consists of woody stem sections attached to tubing, with gravity creating water flow through the stems, and comparing water flow over time to determine which type of wood transports the most water. You can use wood samples cut from any type of shrub or tree. The device can be used as a demonstration, or in real experiments as described in the Advanced concepts and experiment section below. It can also be combined with other lesson plans, as described in the Suggested companion activities section below.

This can be tailored to fit any age or skill level, depending on how much the students participate versus just observing. The time needed to deliver the lesson can range from a few minutes of observation to a full afternoon of lecture and lab work.

Background and significance

Photosynthesis is one of the most prevalent and far-reaching chemical reactions on Earth, and a key component of food production and the global carbon cycle. Most people have a general appreciation for this, and people are also well-aware that plants need water to perform photosynthesis and grow. But most people don't know HOW plants use their water to grow. A small amount of the water plants use is converted to oxygen in the photosynthetic chemical reaction. Most of the water plants use is actually evaporated from cell walls inside the leaf, which are exposed to the dry atmosphere when the plant opens its stomata to gain access to carbon dioxide. The plant water transport system, or vascular system, is critical to photosynthesis because it replaces the water that is lost through evaporation, keeping cells inside the leaves hydrated and functioning. Water transport in the vascular system takes place in specialized cells called xylem, which can be visualized as a bundle of straws inside the wood. The leaves, wood, roots and flowers all contain xylem tissue, inside which there is an unbroken chain of water connecting all of the cells inside the plant to their water source in the soil.

Without the plant vascular system, leaves would quickly dehydrate and die, so the xylem is critical to plant growth and survival. It turns out that plants need to spend a lot of water to keep hydrated: approximately 100-1000 molecules of water evaporate from the leaf for every single molecule of carbon dioxide that is captured. This trade-off between growth and water loss is an important factor in determining plant heat tolerance and drought resistance. When the air is cool and water is unlimited, plants can transport plenty of water to their leaves to keep their stomata open and thus grow rapidly. But when the air becomes very hot or when water is in short supply, water evaporates rapidly from the leaf surface and plants face a choice: 1) they can keep their stomata closed to prevent leaf dehydration, but this stops the flow of carbon dioxide to photosynthesis, effectively shutting down growth and eventually leading to starvation, or 2) they can keep their stomata open to continue photosynthesis, but as the soil and the atmosphere dry out this will eventually cause the vascular system to fail, cutting off the water supply to the entire leaf canopy and allowing the all of the leaves to dehydrate.

This process is taking place every day, in all the plants on Earth, and the amount of water that evaporates from large plant canopies can be enormous. Approximately eight trillion metric tons of water evaporates from the Amazon annually, so plant water transport is a critical component of the global water cycle. In addition, because water transport determines how much the stomata can be opened, and thus how much carbon the plant can obtain through photosynthesis, it represents an important aspect of global food production and the global carbon cycle. In fact, plant water transport is critical for all life on earth. Because plants are the primary producers, their ability to grow determines the resources available for humans and other living things.

The Drip Races brings this under-appreciated phenomenon to life by using a simple flow meter technique to show how wood transports water, how the water transport properties of plants are determined by their wood structure, and the effects of environmental conditions on plant water transport.

Hydraulics flow meters consist of stems attached to tubing, such as this large hydraulics manifold set up with multiple stems. This one is set up as a demonstration of the differences in water transport between different tree species. Red Oak, Live Oak and Avocado were the winners due to their large xylem vessels.

THE DRIP RACES HANDS-ON ACTIVITY

The Goal: Build a measurement device, collect a woody stem sample and get your sample to transport more water, faster than the next person's sample.

This hands-on activity teaches about the physics of plant water transport and the structural and ecological features of species that make them unique and drive biodiversity. Students build a hydraulics measurement device, choose woody stems that they think will "win" The Drip Races by having the highest rate of flow, and run an experiment to see

whose sample will win. Race results discussions focus on interpreting the cause of the measured flow rates from methodological and biological perspectives.

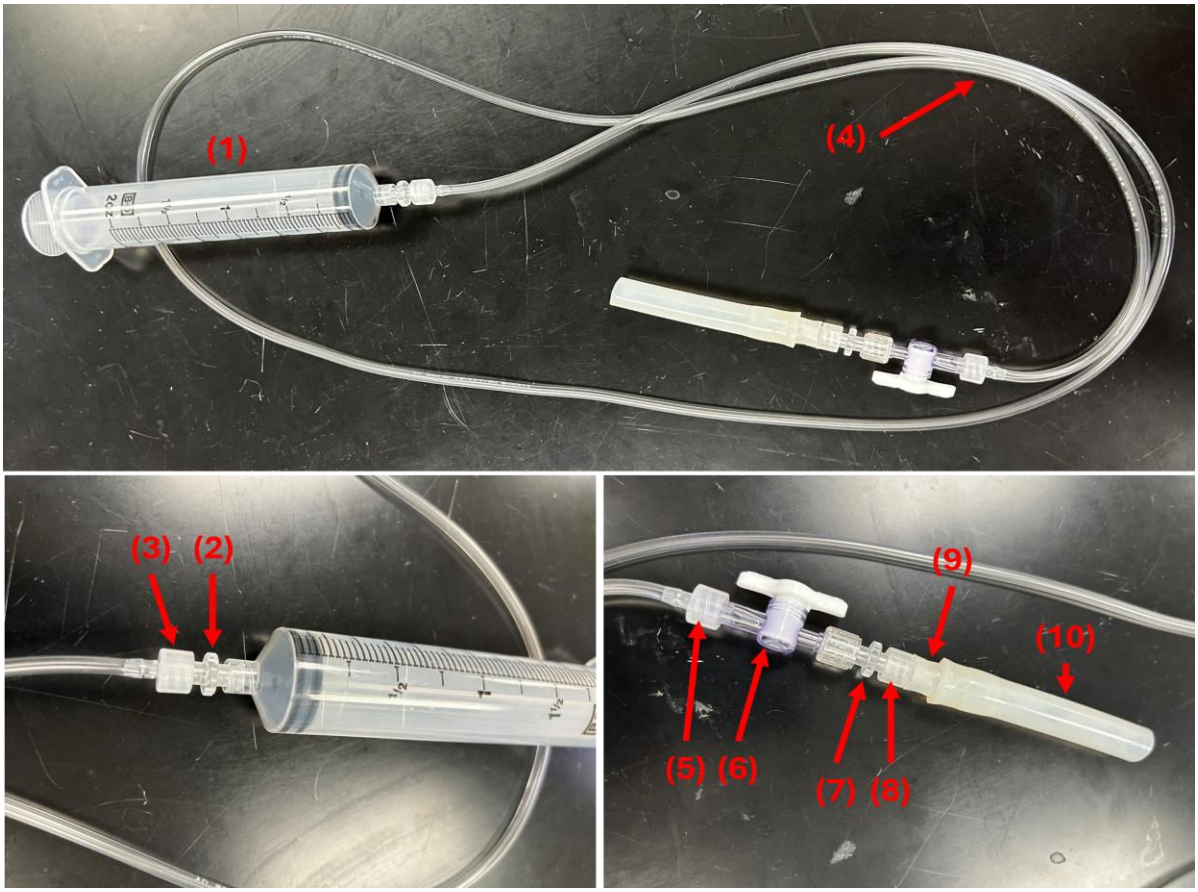
Lab Procedure

On a fundamental level, this is just a flow meter consisting of tubing attached to one or more woody stems. A large manifold like the one shown above can hold many stems and can be used for demonstrations or for real experiments. Below we provide instructions to build a single-stem hydraulics measurement device, but the design can be flexed to accommodate just about any materials or situations. Basically, the water flows from the water source, through the tubing, into one end of the stem and out the other end, due to gravity alone. The rate of water flow through the stem is measured by collecting the water over a time interval and calculating the mL of flow per unit time.

Single-Stem Hydraulics Device Supplies

1. 60mL Luer Lock Syringe, Male fitting
2. Luer Fitting, Female/Female Connector, Straight Union
3. Male Luer to Hose Barb Fittings, Straight Adapter, 1/16" ID
4. (36”) flexible clear plastic tubing 5/32” OD, 3/32” ID, 1/32” wall
5. Male Luer to Hose Barb Fittings, Straight Adapter, 1/16" ID
6. 2-Way Stopcock with Male/Female Luer Connections
7. Luer Fitting, Female/Female Connector, Straight Union
8. Large Bore Male Luer to Hose Barb Fitting, Straight Adapter, x 1/4" ID
9. (2”) flexible silicone tubing 5/16” OD, 3/16” ID, 1/16” wall
10. (6”) flexible silicone tubing 1/2" OD, 5/16” ID, 1/16” wall

The device set up, see below for detailed Suppliers, part numbers and specifications



Step 1. Build a hydraulics measurement device

Attach the syringe (1) to the first female/female connector by screwing the connecter into the end of the syringe (2). Screw on the first hose barb fitting (3). Insert the hose barb into one end of the 36” tubing (4). Insert the second hose barb (5) into the other end of the tubing. Install the 2-way stopcock (6) by screwing the small open end of the stopcock into the open end of the hose barb. Screw on the second female/female connector (7) followed by the large bore male luer (8). Next, insert the large bore luer into the short piece of 5/16” OD tubing (9). Lastly, insert the 6” piece of 1/2” OD tubing (10) over the smaller tubing. The tubing may be sticky and require wetting to make the insertion.

This tubing set up will accommodate sticks approximately 0.4-0.7 cm diameter. For smaller diameter sticks you can use a second piece of the 5/16” OD tubing as an adapter inserted into the end of the larger tubing. Parts list shown at the end of this document.

Step 2. Get water flowing through the device.

Hang the device from a height of 4-6ft. To hold all the tubing and water sources I use ring stands and clamps on a table, with the tubing hanging down onto the floor. This will make a mess with water wherever it is hanging, so place a bucket under the device. You may consider doing it outside. Make sure the stopcock is OPEN (dial is parallel to the direction

of flow), then remove the plunger from the syringe. CLOSE the stopcock (dial is perpendicular to the direction of flow), then fill the syringe with water. Place a wash tub underneath the device and turn the stopcock to OPEN. Water should flow out the end of the tubing, allow water to flow until air bubbles are removed. CLOSE the stopcock and check your device for leaks, use a paper towel to dry the device and then run additional water through. If you find a leak, reattach the components.

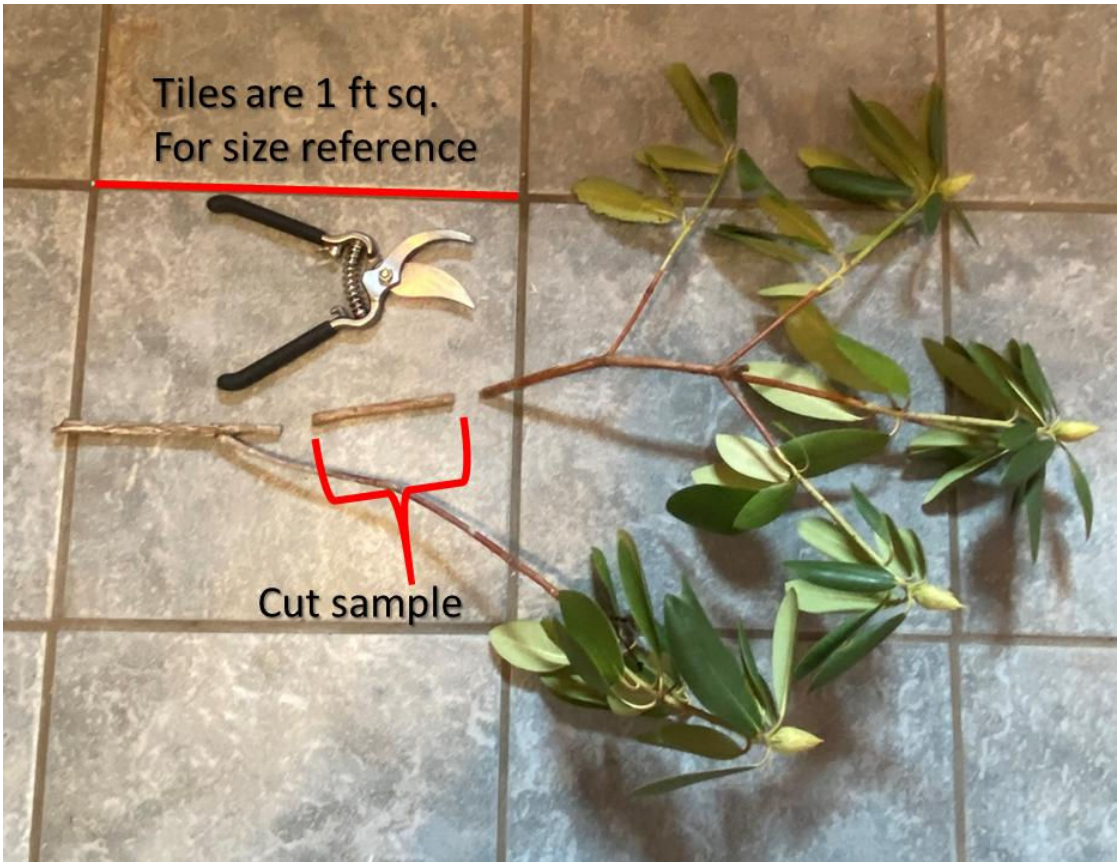
Hang the device from a ring stand and fill it with water.

TIPS: You can use plastic hose clamps to secure any sections of tubing that won't stop leaking. Do not allow the water source to empty at any time, or your device will fill with air. If you fill the device with water, then gently insert the plunger back into the syringe and close the stopcock, the device should remain filled with water and leak free for transport. Pushing on the syringe plunger too much with the stopcock CLOSED will exert pressure on the system and may result in leaks. With the syringe plunger in place, water may not flow even if the stopcock is turned to OPEN.

Step 3a. Collect woody stems.

You can use any live woody stem, but typically it's good to choose plants that will be familiar to your students as this helps them relate to what the wood is doing. Dead wood will not transport water due to decay and clogging by microbes. Choose woody stems of a diameter that will fit snuggly inside your tubing (to prevent leaks). This device is designed for use with stems with diameter approximately 0.4-1 cm. Cut the stems with pruning shears, the sharper the better. It's a good idea to cut a larger branch (1 m), then once in the

lab, trim the sample back from both ends to a length of about 4-10 cm. For your final sample, you'll want to pick a straight section that has no major side branches. You can also trim the sample in the field, but this may result in an unusable sample and taking the whole branch has the advantage of letting you look at the leaf type and total leaf area that was supplied by your stem.



Step 3b. Remove air from samples. This step is optional. Air bubbles inside the stem are a type of damage experienced by wood called an embolism, which blocks water flow to the leaves. These air bubbles will inevitably be introduced into your stem sample when you cut it off the tree. To prevent this, you can collect a larger stem as described above, then once back at the lab, make your final cuts to the sample under water, using a low plastic tub filled with water, place the stem under the water while you make the cuts. This prevents air entry into the stem.

Embolisms can also be introduced during drought and frost events, or by a small insect piercing the surface of the vascular tissue. This is a biological phenomenon and so can be studied by comparing wood samples in their natural state with wood samples where the air is removed. One way to remove air bubbles is to fill a vacuum flask about ¼ full of water and place your stem samples in so they are submerged. Place the flask on a stir plate. Turn on the vacuum, and the air will be pulled out of the stem immediately (you'll have to keep your eye on it to see it). Some stems will produce bubbles for a while. Continue until

bubbles are no longer visibly pouring out of the stem. There are more technical ways to remove embolisms, but the vacuum is a fun way that allows people to see the air come out of the stem.

Step 4. Place samples on device.

Work over the wash tub. With water flowing through the device, hold the tubing so that the open end pointing just slightly up. This will fill the tubing to the very end, with no bubbles. Insert the stick into the tubing with the water flowing, use a twisting motion to insert one side of the stick and then the other. During this step, make sure that the syringe does not empty fully. After inserting the stick, check the tubing for any bubbles. If you see bubbles large enough to fill the tubing, remove your stick and try again. Very tiny bubbles may appear on the side of the tubing, these are usually ok if they don't block the flow. Depending on your tubing and stem, you may need to use plastic hose clamps to stop leaks. Softer, sticky tubing seals well without hose clamps, whereas harder, shiny tubing will likely require hose clamps. Use two pairs of pliers to remove hose clamps.

Technique for loading the sample without introducing bubbles.

Step 5. Measure the rate of flow.

Place a small collection vessel such as a beaker below each stick and use a stopwatch to measure flow rate per minute. Allow the sticks to drip into the collection vessel for 10-30 minutes, then divide the total mL of water by the number of minutes to get the flow in mL/min. Make sure all water flowing into the beaker is coming out of the cut end of your stem. Water flowing around the stem doesn't count! Use a hose clamp to secure the stem if necessary. For more accurate flow measurements, you can use an analytical balance connected to a computer to record either inflow or outflow from the stem.

The sample is attached to the device, use a clamp if water is flowing around the stem, rest the sample in a beaker to collect the water.

Hints for getting the water to flow.

If you set everything up, open the valve and water is not flowing, then you most likely have air blocking water flow somewhere in your system. Check your tubing for air, and re-flush by pouring water through while the system is open. Remove and re-attach your sticks. If you are sure that the tubing has no blockages, it's probably your stem sample that is blocked by air. To alleviate this, you can raise the water source higher, try flushing with the vacuum as described in Step 3b, or try a different plant species. Plants that grow fast and have large vessels are your best bet for producing good water flow.

Suppliers, part numbers and specifications

Total cost per unit $12-$15. All items widely available at web-based and local hardware stores, these suppliers and part numbers are provided as a reference for specifications to ensure you buy items that work together, wherever you purchase.

Item	Supplier	Part #	Cost	Picture	Link
60mL Luer Lock Syringe	Cole-Parmer	UX-07945-42	$53.00/25 pieces		https://www.coleparmer.com/i/cole-parmer-clear-disposable-syringe-luer-lock-tip-non-

					sterile-60-ml-25-bag/0794542
2-Way Stopcock w/Luer Connection	ISM	RAX36-GP0	$1.14/each		https://www.industrialspec.com/shop/medical-products/stopcocks/pvap-mm-pc.html
Female/Female Connector	Cole-Parmer	EW-50114-21	$6.20/10 pieces		https://www.coleparmer.com/i/cole-parmer-luer-fitting-straight-union-crystalvu-cleanroom-packed-female-luer-10-pk/5011421
Male Luer to Hose Barb	Cole-Parmer	EW-50110-11	$6.50/10 pieces		https://www.coleparmer.com/i/cole-parmer-luer-to-hose-barb-fitting-straight-adapter-animal-derivative-free-polypropylene-cleanroom-packed-male-luer-lock-x-1-16-id-10-pk/5011011
Clear plastic tubing 5/32” OD, 3/32” ID	US Plastics Corp	57539	$3.90/10 feet		https://www.usplastic.com/catalog/item.aspx?itemid=74410&catid=864
Large Bore Male Hose Barb Fitting 1/4" ID	Cole-Parmer	EW-50109-60	$21.75/10 pieces		https://www.coleparmer.com/i/cole-parmer-luer-to-hose-barb-fitting-straight-adapter-animal-derivative-free-polypropylene-cleanroom-packed-large-bore-male-luer-

					x-1-4-id-10-pk/5010960
silicone tubing 5/16” OD, 3/16” ID	US Plastics Corp	54032	$11.00/10 feet		https://www.usplastic.com/catalog/item.aspx?itemid=36285&catid=799
silicone tubing 1/2" OD, 5/16” ID	US Plastics Corp	56954	$27.00/10 feet		https://www.usplastic.com/catalog/item.aspx?itemid=134747&catid=799
OPTIONAL Plastic hose clamps (Most commonly used sizes) 0.307" to 0.342" 0.453" to 0.546” 0.513" to 0.586"	US Plastics Corp	57152 57161 57131	$0.30/each		https://www.usplastic.com/catalog/item.aspx?itemid=23427&catid=495

Suggested companion activities

Wood anatomy: The anatomical properties of wood are largely responsible for how water flows in the wood. Specifically, the size of the xylem cells (vessels and tracheids) and the way they are arranged in the stem have a big impact. Investigating the different cell types of wood, and how this differs across species that have different rates of water transport is a fantastic companion activity for the Drip Races. Excellent online resources for this activity include Mauseth’s Plant Anatomy (http://www.sbs.utexas.edu/mauseth/weblab/) and the InsideWood Database (https://insidewood.lib.ncsu.edu/welcome).

Leaf diversity: Activities that invite discussion on the properties of leaves that determine their growth rate and drought tolerance are a good complement to The Drip Races. For example, Oak leaves are thick, waxy and small compared to Maple leaves, so it is easy for people to see that Oak leaves should use less water than Maple leaves. A nice primer on leaf diversity (https://www.ck12.org/biology/leaf-types/lesson/Leaf-Types-Advanced-BIO-ADV/)

Plant identification: Activities that involve observing plant parts to make an identification of species go well with the Drip Races, because in the process students will observe many features that relate to plant function and environmental conditions. An online plant ID key can be found here (https://www.colby.edu/info.tech/BI211/PlantFamilyID.html)

Photosynthesis and leaf anatomy: The Drip Races makes a fantastic complement to lessons on photosynthesis and leaf anatomy because of direct physiological link with water transport. There are many online primers concerning photosynthesis, one of which can be found here (https://opentextbc.ca/biology2eopenstax/chapter/overview-of-photosynthesis/). Mauseth's Plant Anatomy (http://www.sbs.utexas.edu/mauseth/weblab/) is also good for leaves.

Physics of water: The action in the Drip Races is all driven by the physics of water, which has unique properties. Water physics is summarized here (https://w3.marietta.edu/~biol/biomes/water_physics.htm).

Advanced concepts and experiments

Advanced concepts and experiments can be addressed using the same principles and techniques described above. The section "*Catalogs of plant hydraulic methods*" above provides links to detailed protocols for plant hydraulics. The following provides pre-tests that need to be conducted before taking on a full-scale plant hydraulics experiment. Many of the concepts in this section have not been covered in this document, so consultation with the books and on-line resources listed at the end of the document, and the broader scientific literature on this subject is strongly suggested for more complete understanding of proper protocols. Basically there are two main concerns: 1) because plants differ widely in their structure, and because the structure determines vascular function, in order to make comparisons across treatments, your samples will need to be standardized (based on the size, age, position, etc.), and 2) the plant vascular system is dynamic, and anything you do can cause a change in the state of the system, so care must be taken in how samples are collected and handled. You don't have to solve all these problems to get good hydraulics data, but the more you solve, the more accurate the data will be.

What is the xylem structure? Xylem structure (tracheid versus vessels, diffuse versus ring-porous, conduit diameter and length) will influence the magnitude of flow rates and the likelihood of accidentally introducing embolisms during sample preparation. Short stems with lots of large, open vessels will have the highest conductivity because there is less end wall resistance, but these will also refill more easily.

How closely can the size and developmental stage of samples be constrained? The more closely the samples match the better. In most cases samples cannot be perfectly matched, so it will be very important to standardize flow rates in some way, e.g. by sapwood area or by leaf area.

Will any samples have difficult features? Some features, like strong tapering, will make standardization across samples more difficult. Other features, like branch points and absorbent bark, can alter measured out flow rates substantially. If some plants must be excluded because of these features, it is worth considering what the effect might be on the overall results of the study.

Is there evidence for a wounding response to cutting? Sample preparation is a crucial time when artifacts may be introduced. Wounding responses to cutting can reduce or eliminate flow, and they may take 30 mins or an hour to resolve.

What is the native ion concentration of the xylem sap? Perfusion solution impacts the magnitude of flow rates, a perfusion solution of pure DI water will reduce hydraulic conductance. Choosing a perfusion solution that most closely matches the ionic concentration of the sample will reduce the amount of time needed to establish steady-state flow. If treatments are expected to vary in sap ionic concentration, then a single measurement solution should be used for all treatments.

What is the expected range of flow rates? Does the flow rate observed seem reasonable (proper magnitude) given what was expected? Consult with the literature to determine expected flow rates.

How does the expected magnitude of flow compare to the sensitivity of my measurement device? For very low flow rates more sensitive devices, or more time, will be needed to distinguish among treatments. For very high flow rates a less sensitive device will do the job.

What pressure head is sufficient to establish measurable flow? Perfusion pressure should be kept below the point where refilling is occurring. This will depend on the diameter of the conduits, length of the sample vs. conduit length, etc. Having too small of a pressure difference can be a problem because this really lowers the signal to noise ratio. Recommended pressure head is in the range of 1kPa for plants with really large conduit diameters, below 6kPa for those with smaller diameter conduits.

What is the background flow, i.e. without a pressure head, relative to flow at my chosen pressure head? Background flow should be accounted for during each measurement. If background flow is very high compared to the flow established during measurement this could cause high variability in measurements. With higher background flows it may be necessary to make measurements using a larger pressure head (but not large enough to re-fill if native embolism is of interest). Alternatively, measurements can be made over a longer time.

Can steady-state flow be established? Once flow is established in a sample does it remain constant for a good period of time (30 mins)? If flow rates drop over time refilling, wounding, clogging or a response to ionic concentration may be occurring.

How long does it take to establish steady-state flow? If flow rates increase over time, there may be a response to the ionic concentration of your solution.

Are repeated flow measurements on the same sample consistent? Measurements should be repeatable, that is one should be able to return to a sample after

a period (30 mins to an hour) and be able to establish the same flow rate under the same pressure head.

What pressure head is required to refill non-functional conduits? A test demonstrating the minimum pressure at which re-filling is observed is useful for setting an upper bound on the measurement pressure head. Also, if re-filling is part of the experimental design (e.g. for maximum hydraulic conductance) choosing a moderate pressure head that accomplishes refilling will avoid unintended damage to the sample.

How long will elevated pressure need to be applied before refilling is complete? This can be determined by applying pressure, then measuring flow rate, then again applying pressure and measuring flow rate, etc until the measured flow rate does not increase.

How much variation is there among individuals in relation to the variation expected between treatments? This will be important for determining the sample size needed to detect significant differences among treatments.

How much time is needed to make a good measurement at the chosen pressure head? The time needed to make a single flow measurement is in the range of 5 to 60 minutes (depending on the technique, pressure head, species, sample characteristics, perfusion solution, etc.). This will determine the total number of samples that can be measured within the time constraints of the study.

Can the temperature in the lab (or field location) be held constant? Flow rates will depend on the viscosity of water, which changes with temperature.

Are there temporal patterns in embolism formation? Flow rates can change over time in intact plants, for example, some plants can have lower flow rates in the afternoon as compared to the morning. If temporal patterns exist it will be better to confine sampling to a particular time. If this can't be done, then randomizing the time of measurement across treatments can help disentangle time of measurement versus treatment effects.

Resources on plant water transport and plant physiology

On-line primers on plant water transport

https://www.nature.com/scitable/knowledge/library/water-uptake-and-transport-in-vascular-plants-103016037/

https://jplanthydro.org/article/view/25

Catalogs of plant hydraulic methods

Sperry Lab http://sperry.biology.utah.edu/methods.html

Jacobsen Lab https://www.csub.edu/~ajacobsen/Research_Methods.htm

Books about plant physiology and water transport

Nobel, PS. 2009. Physicochemical and Environmental Plant Physiology. 4th edition. Amsterdam, Academic Press.

Tyree, M.T. and M.H. Zimmermann. 2002. Xylem Structure and the Ascent of Sap. Berlin, Springer-Verlag.
Carlquist, S. 2001. Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Berlin, Springer-Verlag.

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