Chapter 13

Methods for Xylem Sap Collection

M. Alexou and A.D. Peuke

Abstract

Xylem and phloem are essential for the exchange of solutes and signals among organs of land plants. The synergy of both enables the transport and ultimately the partitioning of water, nutrients, metabolic products and signals among the organs of plants. The collection and analysis of xylem sap allow at least qualitative assumptions about bulk transport in the transpiration stream. For quantification of element-, ion-, and compound-flow, the additional estimation of volume flow is necessary. In this chapter we describe methods for collecting xylem sap by (1) root pressure exudate, (2) Scholander-Hammel pressure vessel, (3) root pressurizing method according to Passioura, and (4) (hand/battery) vacuum pump.

Key words: Xylem sap, Root pressure, Scholander-Hammel pressure vessel, "Passioura vessel"

1. Introduction

One of the most important and characteristic features of higher plants is their adaptation to life on land via the evolution of long distance transport systems. The requirement of long distance transport is fulfilled by phloem and xylem in cormophytes, which enable the transport of water, nutrients, and signals among the organs of plants (1). In the xylem water, minerals, products from root metabolism, and signals are transported from the root to transpiring parts of the shoot, particularly the photosynthetically active leaves. Large and especially tall plants must exhibit special features in their xylem. For example, tall trees (up to 100 m in height) must overcome significant gravitational forces in order to lift transport saps to the top of the tree.

The current view of the driving forces of long distance transport is based on gradients in the transport systems, i.e., gradients in hydrostatic pressure, water potential, and chemical potential. Gas exchange (water vapor, CO_2, O_2) and associated processes are central factors in regulating the long distance transport.

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Regardless of recent criticism on the common hypothesis for long distance transport, there is undisputed agreement that in the xylem there are complex gradients in pressure and potential (2). This results in difficulties in the investigation of xylem function, especially when conducted *ex situ*, since manipulation can lead to significant artifacts, particularly in quantification of fluxes. The knowledge of xylem sap composition is, therefore, a very important step in understanding plant nutrition and stress conditions of plants (1).

In transpiring plants, xylem sap is usually under tension, rather than under pressure, due to the suction of the transpiration stream (2). For collection of xylem sap this negative pressure has to be compensated and overcome. We present four simple methods for xylem sap collection, most of which can also be applied in the field: (1) root pressure exudation, (2) Scholander-Hammel pressure chamber, (3) root system pressurizing chamber according to Passioura (1980), and (4) hand- or battery-operated vacuum pump.

1. The root pressure method can be applied in some cormophytes by removing the transpiring shoot of a plant near the root–shoot interface. Xylem sap will be exuding after some minutes from the cut stem for hours or even days (depending on species and conditions) due to root pressure. The phenomenon of root pressure is the outcome of active transport of mineral ions and passive inflow of water into xylem vessels of the root. Root pressure provides the driving force to transport the xylem sap some distance up the stem of a decapitated plant. Root pressure can also be observed in springtime, short before bud break, and is used for xylem sap collection. Stored starch in pith rays is degraded and the generated sugars are loaded into the xylem vessel resulting in root pressure. This mechanism can be seen in the case of pruned grapevines (see Fig. 1c) or in sugar maple, where it forms the basis for collecting the feedstock for maple syrup.
2. Xylem sap can also be collected using a modification of the "pressure vessel technique" of Scholander et al. (3). According to this method, gas pressure is applied to a plant part/twig to compensate the negative pressure in the xylem vessels and thus cause xylem sap flow in the opposite direction. It is usually used for tree and bush twigs and roots, which once cut from the stem, must be brought to the Scholander device as soon as possible. This device consists principally of a vertical metal cylinder (50 cm long depending on the model) on a bank with its upper end open and its lower part tightly fixed on the bank. The cylinder is supplied with N_2 from a gas cylinder fixed at the side of the device. The cylinder can be tightly sealed with a special cup, in the middle of which the twig (or root) is appropriately placed for the sampling purposes.

3. The Passioura—method for collection of xylem sap (4) involves applying pneumatic pressure to the root system in order to overcome the xylem tension. This method can be used in the lab or the greenhouse only. Xylem sap can be collected from pressurized root systems using a pressure vessel technique, from cut midribs of leaves, flaps of stem tissue or petioles of otherwise intact plants. Plants used for measurement must be grown in special pots, which are sealed into the pressure chamber at the hypocotyl junction with dental silicone impression material. The entire root system is pressurized to 0.2–2.0 MPa, sufficient to cause flow through the xylem system from the roots at rates just exceeding shoot transpiration, thereby causing exudation from various sampling points. This method is nondestructive, since xylem saps are collected from living plants, which do survive the procedure.
4. The vacuum pump (5) is used when higher amounts of xylem sap are assumed in the collected plant material, i.e., in cases where plants are not xerothermic and the environmental moisture is increased. The (hand-) vacuum-creating device is like a small portable pistol and the procedure is simple. However, it takes two persons to operate it. The advantage of this method is the low weight and low cost of the vacuum-creating device without the need for other equipment.

2. Materials

2.1. Materials for "Root Pressure Method"

Gardner scissors, knife, scalpel, distilled water, silicon tubes (diameter depending on experimental plant), silicone grease, plastic vials/eCups, pipettes.

2.2. Materials for "Scholander-Hammel Pressure Vessel Method"

Gardner scissors, scalpel, distilled water, single-use paper towels, plastic vials, pipettes/Pasteur pipettes (150 mm, VWR International), N_2 gas bottle. Scholander devices are offered by a number of companies, for example, UMS GmbH, München, Germany, (http://www.ums-muc.de) or MMM—Mosler Tech Support, Berlin, Germany (http://www.mmm-tech.de).

2.3. Materials for "Passioura Pressure Vessel Method"

Self-made pressure vessel, tool kits (like screwdrivers, screw wrenches, spanners), knife, scalpel, distilled water, Teflon tubes (diameter depending on experimental plant), silicone grease, dental silicone impression material (e.g., hydrophilic vinyl polysiloxane), correction material (blend-a-gum, light N, normal setting: blend-a-med Forschung, Schwalbach, Germany), plastic vials, pipettes, N_2 or pressurized air bottle.

2.4. Materials for "Vacuum Pump Method"

Gardner scissors, scalpel, distilled water, single-use paper towels, plastic/glass vials, (bung, funnel, *for option 1*), rubber tubes, pipettes/Pasteur pipettes (150 mm, VWR International). The vacuum pump can be made of plastic or zinc and can be acquired from companies such as Shreveport Air Tool, Inc. Los Angeles, USA (http://www.tooltopia.com), Fisher Scientific UK Ltd Leicestershire, UK. (http://www.fisher.co.uk), or Environmental Express, Charleston, South Carolina, USA (http://www.envexp.com).

3. Methods

3.1. Root Pressure Method

1. Supply the plant with water or nutrient solution before sampling in order to create less negative water potential in the soil and facilitate water uptake.
2. Cut the plant at the root–shoot interface, to remove all transpiring leaves.
3. Remove 1–2 cm of the bark (if present) below the cut site.
4. Clean the cut surface with deionized water.
5. Fit a silicon tube to the shoot stump (Fig. 1a), if necessary seal with silicone grease.

Fig. 1. Illustrations from the root pressure method. (**a**) A simple experimental setup for xylem sap sampling. (**b**) A grapevine cane with bud, shortly before bud breaking. (**c**) Xylem sap (*arrow*) exudation shortly after cutting the cane in spring time.

6. Wait for the appearance of the first root pressure sap, some few minutes up to several hours after decapitation, depending on the conditions of the plant or the rhizosphere conditions.
7. Remove the very first μLs to avoid contamination from cut tissue/cells.
8. Collect the appearing xylem sap with a pipette and store it in plastic vials on ice until the needed volume is reached; subsequently freeze the samples.

3.2. Scholander-Hammel Pressure Vessel

1. Cut off a twig from the tree/bush with clean gardener's scissors. The most appropriate diameter of a twig is between 0.5 and 1 cm. Make sure the total length of the twig does not exceed the length of the cylinder of the device, so that it will not fold inside the cylinder, since the slightest damage of the twig must be avoided.
2. Remove carefully an adequate extend of the bark (at least 1 cm long) of the twig from the vicinity of the cut surface, to avoid contamination of the xylem sap.
3. Clean up the cut end of the twig with distilled water.
4. Place the twig vertically into the cylinder, so that the cut end protrudes 2–3 cm out of the upper/open end of the cylinder (Fig. 2a "ts" and "cy"; d).
5. Pass the cut end through the cylinder cup (Fig. 2a "sc"), and through its accessory, the appropriate rubber hole, which sits in the middle of it and is arranged to be extremely narrow for the twig under examination (Fig. 2a "rt").
6. The cylinder cup is placed onto the upper end of the cylinder with the twig emerging from the center of it (Fig. 2a "cy"). The cup is screwed gas-tight to the cylinder, while the twig is immobilized on the screw cup with a second, smaller, screw cup placed externally upon the cylinder cup (Fig. 2a "es"). Make sure once more that the cut end of the twig that emerges out of the cylinder cap has adequate length, since this procedure may decrease the length of the twig.
7. Make sure the cylinder cup is tightly screwed on the cylinder and the external screw cup tightly screwed on the cylinder cup, so that the friction between twig, rubber, and cylinder cup makes it impossible for the twig to move and the N_2 to escape from the vessel (Fig. 2a "tc").
8. Safety glasses must be put on and distance must be kept between one's head and the upper part of the cylinder at all times when operating the device.
9. Having assured that the system is sealed and the valve for pressure release is off (Fig. 2b "vr"), turn the pressure inlet valve on the side of the bank (Fig. 2b "vi") gently, just enough to

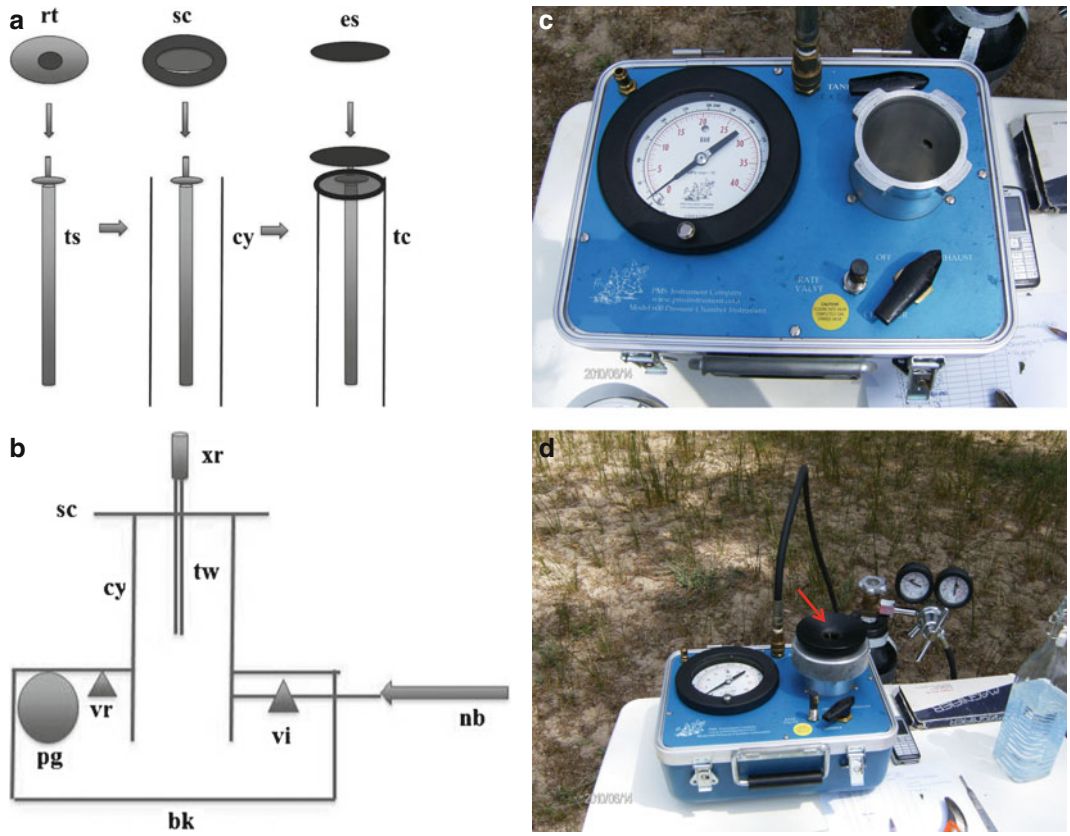

Fig. 2. Illustrations from Scholander pressure vessel method. (**a**) Scheme presenting twig placement. (**b**) Scheme presenting basic traits of Scholander device, bk: bank; cy: cylinder of Scholander device; es: external screw cup securing twig in first screw cup; nb: N_2 bottle; pg: pressure gauge; rt: rubber for twig immobilization; sc: screw cup adjusted to rubber, securing the cylinder closure; tc: twig secured within cylinder; ts: twig during sampling; tw: twig; vi: valve for N_2 inlet; vr: valve for N_2 release; xr: xylem sap concentration rubber. (**c**) Open Scholander device without twig. (**d**) Scholander device connected to N_2 bottle. From the inside of the twig's cut end (*see arrow*) xylem sap emerges.

allow a small amount of N_2 (Fig. 2b "nb") to enter the cylinder. Carefully follow the rise in pressure and then turn off the pressure inlet valve to stabilize the pressure to a low level.

10. The rate of pressure increase should not exceed 50 kPa/s. Do not let N_2 flow into the cylinder for longer than 5 s at a time. Turning it off after short time intervals and monitoring closely the rise of pressure (Fig. 2b "pg") (even after the valve is turned off) is necessary. On the other hand, if the pressure in the cylinder takes long to increase (>10 s N_2 flow), or falls at a rate of more than 50 kPa/s, leakage of the system is likely.
11. The desired pressure is achieved, when xylem sap emerges at the cut surface. The appearance of xylem sap is evident when the color of the cut twig surface darkens and tiny drops with air-bubbles appear.

12. This pressure value is regarded as the apparent shoot water potential of the twig.
13. Discard the first drops of xylem sap to avoid contamination with tissue content. Put a clean, thin, open-ended rubber tube onto the twig (Fig. 2b "xr"), in order to facilitate the accumulation of continuously emerging xylem sap.
14. Raise the pressure above the shoot water potential and maintain this pressure by adding or interrupting the N_2 flow, so that a stable flow of sap is achieved. It is not advisable to let xylem sap flow beyond what is needed for collection. For additional volume of sap one must add N_2 in order to sustain or even increase the pressure within the cylinder.
15. The pressure should not exceed 3.5 MPa because increasing the pressure on the twig tissues will cause cell and tissue damage which might contaminate the xylem sap.
16. Collect the xylem sap using single-use Pasteur pipettes, or automatic pipettes used in biochemical laboratories (most effective are the tips of <20 µl).
17. Transfer the xylem sap to plastic vials and subsequently freeze the samples. The samples must be stored frozen until analysis.
18. After completing the sampling of the xylem sap, open the pressure release valve in order to achieve balance with ambient pressure. Leave enough time for the cylinder to empty, always watching the pressure until it falls to zero, and then unscrew the cylinder cup to replace or discard the twig.

3.3. Passioura "Pressure Vessel": Rhizosphere Pressurizing

1. Construct a pressure chamber from stainless steel (Fig. 3a/b).
2. Grow plants in plastic vessels (Fig. 3a "gv") fitting into the pressure chamber.
3. Supply the plants with water or with nutrient solution.
4. Before transferring the plastic pots to the steel pressure chamber, suck off the excess water, for example, on a tension table, at 5 kPa suction.
5. Place the plant in the middle of the pressure vessel; test the position with the twice-splitted top cover (Fig. 3 "tc") clean and grease the O-sealing ring (Fig. 3 "os").
6. Glue the twice-splitted top cover with dental silicone impression material at the V-formed interface and subsequently screw together the two halves.
7. Screw the merged top cover on the pressure vessel with the fixture (Fig. 3 "tf").
8. Seal the plant stem with dental silicone impression material in a way that a glue plug is formed above and below the top cover to ensure the vessel is gas-tight (Fig. 3 "gp").

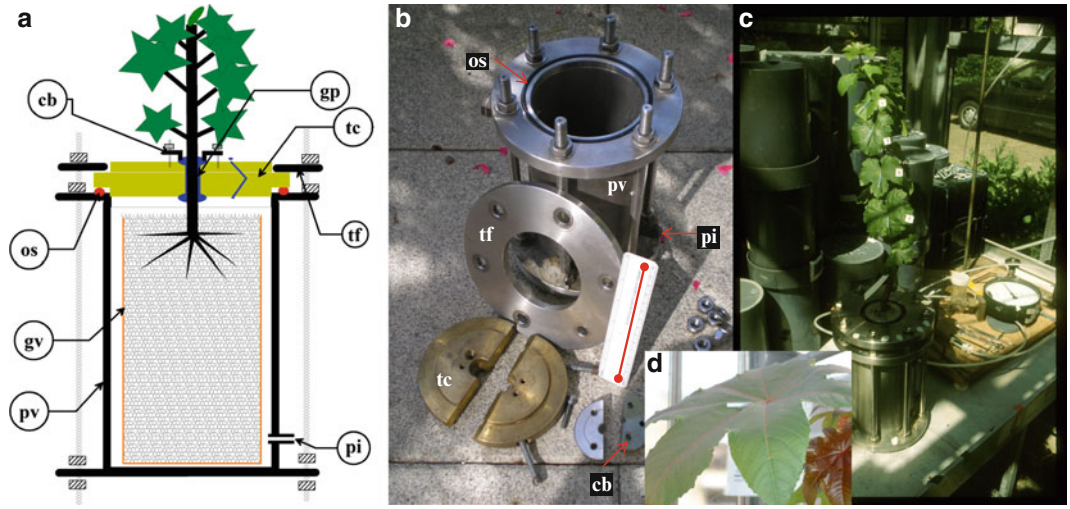

Fig. 3. Illustration from Passioura pressure vessel method. (**a**) Scheme of the Passioura pressure vessel. (**b**) The single components of the vessel. (**c**) The entire system with grapevine, sap from seven individual leaves was collected. (**d**) Teflon tube fitted to the cut midrib of a *Ricinus* leaf. cb: counter bearing; gp: glue (impression material) plug; gv: growing vessel; os: O-ring seal; pi: pressure inlet; pv: pressure vessel; tc: twice-splitted top cover; tf: top cover fixture. The bar in (**b**) indicates 15 cm.

9. Fix the bearing support (Fig. 3 "cb") on top of the stem in order to stabilize the glue plug between the stem and the twice-splitted top cover.
10. Cut potential xylem strands of interest, for example, midrib of leaves in the middle of the laminae (Fig. 3d) or flaps from stems, and fit a rubber to the basal part of the dissected midrib. Several leaves can be cut in the same plant and sap can be sampled simultaneously (Fig. 3c).
11. Tightly fit a tube to the cut xylem strands/midrib, if necessary by additional sealing with silicone grease.
12. Safety glasses must be put on and distance must be kept between one's head and the upper part of the cylinder.
13. Increase the pressure in the root system stepwise at a rate of 0.1 MPa per 10 min, by releasing N_2 into the pressure vessel.
14. The compensation pressure at which the first xylem droplets appear at the cut xylem strands/midrib is recorded as the local apparent water potential.
15. Discard the first µLs of sap to avoid contamination from cut cells and tissues.
16. Increase the pressure to 0.1–0.2 MPa above the compensation point of the sampling threshold. Pressure can be applied in the range of 0.5–2.0 MPa to obtain sufficient flow from all cut sampling points simultaneously.

17. Collect the saps from the fitting rubber tubes (Fig. 3d), store them temporarily on ice, weigh them, and freeze immediately.
18. By determining the volume of collected xylem sap samples (i.e., by weighing the cup before and after sampling) compared with the time for sap collection, the relative apparent volume flow of the different sampling points is estimated.

3.4. Vacuum Pump

1. Prepare twigs as in the description of the Scholander device. There are two options apart from the basic principal (Fig. 4a). First option:
2. After having removed the surrounding bark, tightly place the cut end of the twig (Fig. 4b "tw") in a collection vial (Fig. 4b "cv").

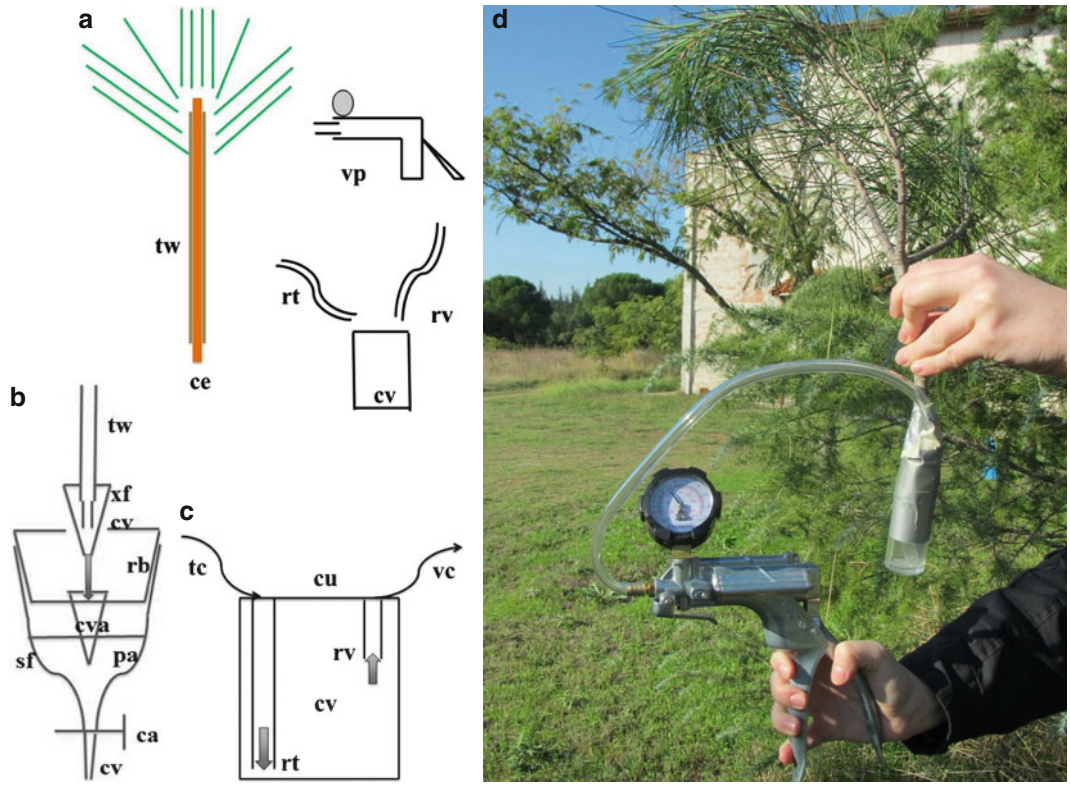

Fig. 4. Illustration from vacuum pump. (**a**) Scheme presenting basic arrangement for sampling. (**b**) Scheme presenting the first option to implement the method. (**c**) Scheme presenting the second option to implement the method. ca: cannula; ce: cut-end prepared; cu: air proof cup of collection vial; cv: collection vial; cva: collection vial, appropriate position; pa: packing holding vial supporting vacuum; rb: rubber bung; rt: ((**c**) ending of-) rubber connecting twig to collection vial; rv: ending of rubber from vacuum pump; rv: rubber connecting vacuum pump to collection vial; sf: separating funnel; tc: twig connection (air proof); tw: twig (progressively trimmed); vc: vacuum pump connection (air proof); vp: vacuum pump; xf: xylem free from bark. (**d**) Photograph of the arrangement (option 2 of the described method) using a glass-vial attached to the vacuum pump and the twig, while being sealed air-tight with packing and duck-tape.

3. Adjust the collection vial for the xylem sap to the rubber bung (Fig. 4b "rb").
4. Fix the vial and the rubber bung on a larger funnel filled with packing to hold the vial and support vacuum (Fig. 4b "sf").
5. Adjust the lower end of the funnel to an opening where the vacuum pump is fixed (Fig. 4b "cv").

Make the arrangement air-tight (Fig. 4b "cva"), and create vacuum with the vacuum pump. Full blank port pressure can be obtained with only two strokes. One stroke is enough to create a vacuum of −15 kPa and enough for the xylem sap to start flowing.

6. As the first drops of sap appear and the first person holds the twig's end and operates the pump (manually or battery functioning), a second person cuts the upper part of the twig with a gardener's scissors in a stepwise manner, so that further drops flow gradually into the vial.
7. After the sampling, the vacuum is dissolved using the cannula (Fig. 4b "ca"), or the appropriate valve on the pump, the arrangement is dismantled, the bung and the funnel are cleaned, and the twig is replaced.

The second option is to use an air-tight trap (a vial) between the twig and the vacuum pump, ideally using a cup with two tube connections:

8. This arrangement leads the xylem sap from the twig flowing through a rubber tube (Fig. 4c "tc") into the vial (Fig. 4c "cv").
9. The vacuum is created by a vacuum pump attached to the same vial by a second tube that connects the cup of the collection vial and the vacuum pump (Fig. 4c "vc"). Full blank port pressure can be obtained with only two strokes. One stroke is enough to create a vacuum of −15 kPa (Fig. 4c "rv") and enough for the xylem sap to start flowing (Fig. 4c "rt"). As the first drops of sap appear and the first person holds the twig's end and operates the pump (manually or battery functioning), a second person cuts the upper part of the twig with a gardener's scissors in a stepwise manner, so that further drops flow gradually into the vial. After the sampling of the xylem sap, the vacuum is released by the appropriate valve of the vacuum pump and both the twig and the vial are replaced, whereas the cap is cleaned.
10. A simpler way for this arrangement to work is to replace the vial with the cup by using a plastic/glass vial for sampling, into which both rubber tubes are inserted (Fig. 4d). The tube leading to the vacuum pump is placed higher into the collection vial than the one connected to the twig. Both are fixed very tightly. The vial with the two tubes is sealed with air-tight duck-tape.

4. Notes

Root pressure method

1. This method works best with plants having big xylem vessel diameters.
2. Do not use on plants under stress, like salinity, nutritional disorder or even drought.
3. Do not collect xylem sap from decapitated transpiring plants for longer than needed for sufficient volume, because recycled compounds derived from the phloem transport will be excluded and energy reserves in the roots for active loading of ions into the xylem vessels only has limited capacity.

Scholander-Hammel pressure vessel

1. In order to complete a sampling task in a preordained amount of time, it is often the case that a number of twigs must be sampled in a short time. It should be considered that the time needed for the sampling of xylem sap from a single twig can vary considerably depending on the experience of the operator, the xylem sap content of the twig and the environmental conditions.
2. In order to ensure stability of compounds in the collected saps, it should be considered that the sap should be aliquoted into multiple vials, since (particularly in the case of amino acid measurements) frozen sap should not be subjected to more than two freeze-thaw cycles.
3. It is risky to try to extract xylem sap from a wet twig in the field. In extreme cases, like on rainy days, first carefully wipe the twig with a clean tissue, while ensuring no mechanical damage which could cause xylem sap to escape from non-visible parts. If the moisture has penetrated the twig, the Scholander device could detach the bark from the woody tissue. The increased pressure could explosively eject the twig out from the pressure vessel and injure the person operating the device seriously.
4. Extreme pressure (>3 MPa) must be avoided due to the existing danger of exceeding the upper pressure limit of the cylinder's capacity, leading to explosive ejection of the cylinder cup.
5. Another case when ejection of the cylinder cap can occur is when very high pressure is applied to collect xylem sap. This can happen when the water potential of the experimental plants is very negative, for example, after a long period of drought or under saline conditions. For this reason always keep an eye on the pressure applied and avoid the maximal capacity of the vessel.

6. The cylinder cap can also be explosively ejected when it is not tightly or carefully screwed in place.
7. The time to put the Scholander device together should always be taken into consideration when planning a field campaign. It is advised to train the experimenter before hand with a similar sized twig. A spare bottle of N_2 should always be available, because the Scholander device can consume high amounts of N_2 when used extensively.

Passioura "pressure vessel": rhizosphere pressurizing

1. Xylem sap can normally not be obtained from the oldest or youngest leaves of a shoot.
2. Plants with aerenchyma are also not appropriate for the Passioura pressure vessel, since the collapse of the aerenchyma may occur under high pressure, creating a direct connection with the apoplastic space.
3. Contamination of the xylem sap with phloem is avoided by this method; no sugars are detected and the pH of the sap is substantially lower than 7.
4. Solute concentrations decrease hyperbolically when pressure increases in the root system and therefore flow rates increase.
5. The highest solute concentration can be recorded under root exudation conditions, while the lowest values were recorded under pressurizing conditions (6).

Vacuum pump

1. In both arrangements the vacuum itself is not easy to sustain.
2. In case the vacuum pump is not battery-powered, it can be quite difficult to operate the vacuum pump for a longer time.
3. One should never try to use the vacuum pump directly connected to the twig in the hope of collecting xylem sap drops from the connection rubber itself. The vacuum pump will be severely damaged.

Additional methods

1. Xylem sap of stem sections of trees can be collected by a decompression method developed for the mechanical drying of timber (7).
2. A continuous, nondestructive method for sampling xylem sap in intact transpiring plants uses the xylem-feeding insect meadow spittlebug (*Philaenus spumarius* L. (Homoptera: Cercopidae)) (8).
3. Small volumes of xylem saps can also be collected with a pressure probe (2).

Storage and analysis of xylem sap

1. Prior to analysis xylem sap should be stored frozen. Depending on the compounds of interest the freezing temperature can be –20°C in case of inorganic ions down to –80°C for amino acids, thiols, hormones, etc.
2. Xylem sap is an aqueous solution and can be easily used after dilution for appropriate analytic methods.
3. Xylem sap in vials often consist of tiny drops which can be difficult to pick up even with the thinnest pipette. It is strongly advised to centrifuge sample vials at 4°C for 2–3 min to coagulate all liquid.
4. Analysis of xylem sap usually involves amino compounds, ionic compounds, thiols, and hormones (e.g., abscisic acid). While necessary precautions must be taken in all cases, proper storage and handling is especially important when organic compounds such as amino acids, thiols, and hormones are of interest.

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