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Monitoring Xylem Hydraulic Pressure in Woody Plants

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[Abstract] Xylem sap circulates under either positive or negative hydraulic pressure in plants. Negative hydraulic pressure (*i.e.,* tension) is the most common situation when transpiration is high, and several devices have been developed to quantify it accurately (*e.g.,* Scholander pressure chamber, psychrometers). However, a proper measurement of positive xylem sap pressures may be critical when pressure is generated by the root system, allowing vessels to be refilled. Here, we describe two different methods to monitor positive xylem bulk pressure: the pressure gauge which can only be set onto a rootstock or a side branch and the point pressure sensor, which can allow measurements from a functioning plant without detopping or cutting.

Keywords: Pressure, Tension, Water status, Xylem water potential

[Background] Although plants can recover from critical levels of xylem embolism, < 50% loss of hydraulic conductivity in conifers (Brodribb and Cochard, 2009) and < 88% in angiosperms (Urli *et al.*, 2013), the exact mechanism is still under debate. The ascent of sap is driven by the evaporative demand from the atmosphere, which generates a negative pressure (*i.e.,* tension) in the water column and hydrogen bonds between molecules (*i.e.,* cohesion) pull the sap through the plant via the well accepted cohesion-tension theory (Dixon, 1896; Angeles *et al.*, 2004). However, positive xylem sap pressure can be recorded under particular conditions, for example water-saturated soil combined with very low transpiration. This mechanism has been shown to refill embolized vessels in springtime (Sperry *et al.*, 1994) and in species that experienced freeze-thaw induced embolism (Charrier *et al.*, 2013 and 2014). Refilling of embolized vessels has been hypothesized to occur under both positive and negative xylem sap pressures in *Laurus sp* or *Vitis sp*, for example (Salleo *et al.*, 1996). However, the 'refilling under tension' mechanism is inconsistent with the cohesion-tension theory (Zwieniecki and Holbrook, 2000). Moreover, recent works suggest that refilling occurs only under positive pressure in *Vitis* (Charrier *et al.*, 2016). The dynamic changes in xylem sap pressure therefore need to be explored at both the seasonal and diurnal scale while maintaining as much as possible the integrity of the hydraulic architecture of the plant.

Although the use of stem psychrometers has been extensively described since the 80's (*e.g.,* Dixon and Tyree, 1984; Tyree and Dixon, 1986), the measurement of positive xylem sap pressure is relatively

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rare. The protocol described here allows the quantification of the spatio-temporal pattern of bulk xylem sap water potential under positive pressures, and even moderate tensions (maximum of 0.05 MPa) along the water column using non-invasive sensors (*i.e.,* point pressure sensors).

Materials and Reagents

1. Parafilm M (Bemis, catalog number: PM996)
2. Stainless-steel hypodermic needle 21 G 1 ½" (Terumo Medical, catalog number: 8AN2138R1)
3. Union–1/16" PEEK (Interchim, catalog number: 869290)
4. Lock ring (Ark-Plas Products, catalog number: LEX66-PP0)
5. Threaded male Luer connector 10-32 UNF (Ark-Plas Products, catalog number: LGX74-PP0)
6. Reinforced PVC flexible tubes (RS Components, catalog number: 440-874)
7. Zip ties *e.g.,* RS Pro Black Nylon Non-Releasable Cable Tie, 300 x 4.8 mm (RS Components, catalog number: 233-487)
8. 4-way Luer Lock Stopcock, Male-Male-Female (Cole-Parmer, catalog number: EW-30600-04)
9. Stainless steel high quality single edge blades (*e.g.,* Mure & Peyrot, catalog number: 144.3)
10. Nylon Hose clips (RS Components, catalog number: 291-587)
11. Cutting disk (RS Components, catalog number: 448-7439)
12. HSS Drill bit, 0.8 mm diameter (*e.g.*, RS Components, catalog number: 457-651)

Note: Most parts are available from the laboratory equipment suppliers.

Equipment

1. High resolution datalogger (*e.g.,* Campbell Scientific, model: CR1000)
2. Pressure transducer 30Psi (Honeywell International, catalog number: 26PCDFA6D)
3. Stabilized power supply 12V DC (*e.g.,* Traco Power, catalog number: TML 20212C)
4. Hand-held driller

Procedure

Directly attaching a pressure sensor at the distal end of a cut stem (*i.e.,* pressure gauge) allows quantitative measurement of the pressure however at the cost of removing the upper part of the plant. Another approach, less interfering but still invasive (*i.e.,* point pressure sensor), is to connect the pressure sensor to the xylem via a stainless steel hypodermic needle (Clearwater *et al.*, 2007; Thitithanakul, 2012, see Figure 1). The latter allows plant functions (*e.g*., transpiration) to continue and pressure to be recorded at different heights along the water column. Xylem sap pressure is measured by a pressure transducer connected to a datalogger that records the output signal (positive or negative). The connection between the sensor and the xylem has to be perfectly sealed using Parafilm in order to accurately estimate the pressure.

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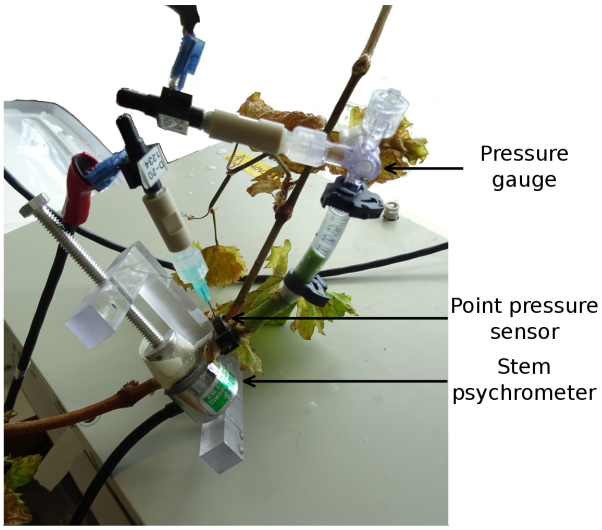

Figure 1. Distal part of a grapevine plant (*Vitis vinifera* cv. Grenache) showing examples of the three distinct instruments. From bottom to top, stem psychrometer PSY-1, point pressure sensor and pressure gauge.

1. Preparation of the sensor (Figure 2)

   Wrap the threaded end (10-32 UNF) of the pressure transducer (#1 Figure 2) and the male Luer connector (#3 Figure 2) with Parafilm and screw up at both ends of the union 1/16” piece (#2 Figure 2). Place the Luer lock (#4 Figure 2) onto the male Luer connector. Fill the sensor with deionized degassed water to remove all air bubbles.

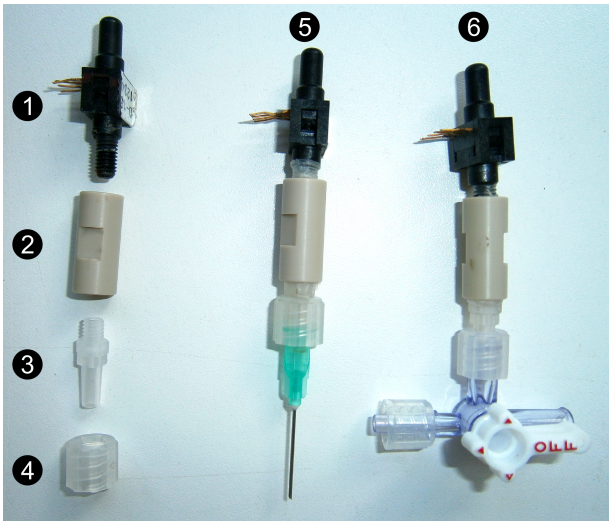

Figure 2. Different parts and assembly of the sensors. Pressure transducer (1), union 1/16” piece (2), male Luer connector (3) and Luer lock (4), point pressure sensor (5) and pressure gauge (6).

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a. Pressure gauge (#6 Figure 2)

   Connect a 3 cm-long piece of adapter tubing to the 4-way Stopcock. Wrap the region of interest of the distal end of the stem with Parafilm, cut the stem 2-3 times under water with a sharp blade. Remove bark and cambium over 1 cm using the blade and immediately insert the cut end into the adapter tubing, tighten with a collar. Fill the whole system up with deionized and degassed water to remove all air bubbles using a syringe.

b. Point pressure sensor (#5 Figures 2 and 3)

   i. Using a small disk, gently make a notch 2.0 cm from the base of the stainless steel hypodermic needle (21 G 1 ½”; #1-2 Figure 4), and cut the needles at 2.1 cm. Remove all barbs and make sure that the needle is not plugged using a needle of smaller diameter . Drill a hole of the exact diameter of the needle 1 cm away from the lock of the zip tie. Insert the needle into the hole and wrap the basal 2.0 cm with Parafilm (#3 Figure 4).

   ii. Drill a hole of the exact diameter of the needle in the stem using a drill bit (0.8 mm diameter for 21 G 1 ½” needles). Pierce to the xylem and then wash it with deionized water. Gently insert the stainless steel hypodermic needle, previously filled with water, into the hole. The notch of the needle must locate within the xylem (Figure 3). Tightly fix the needle with the zip tie.

Figure 3. Point pressure sensor inserted into a grapevine plant. The frame illustrates the cross-section of the stem and shows the insertion of the needle into the stem xylem.

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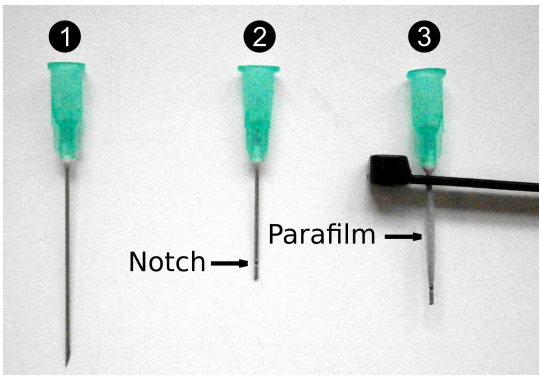

Figure 4. Preparation of the needle used for the point pressure sensor. The stainless steel hypodermic needle (1) is cut and notched (2). The needle is inserted into the zip tie and wrapped with Parafilm (3).

2. Connection of the sensor (Figure 5)
   Fill both pressure sensor and inserted needle with deionized degassed water (#1 Figure 5), and screw them together (#2 Figure 5). Connect the pressure transducer to the datalogger, with stabilized 10 or 12 V (D.C) input voltage.

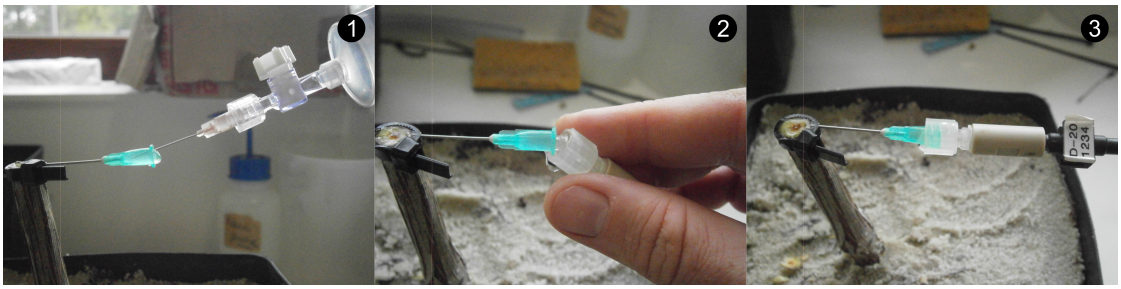

Figure 5. **Connection of the point pressure sensor.** Once the inserted needle is filled with deionized and degassed water (1), the sensor is screwed onto the needle (2, 3).

Data analysis

1. Each pressure transducer has a slight offset from atmospheric pressure. It is therefore recommended to measure the output signal (the offset U_0) for a short period of time (*e.g.,* > 5-10 measurements by the datalogger) before any measurement (calibration or connecting the pressure transducer to the xylem of a plant).
2. Calibration can be performed using a pressure gauge to measure the output signal along the normal operating range of the sensor (*e.g.,* 30 Psi [*ca*. 0.2 MPa] for 26PCFFA6D sensors; Figure 6). The output tension (U_{out}) depends on the input tension (U_{in}) and the calibration coefficient of the sensor. It therefore should be normalized if U_{in} is not stable along the experiment (*e.g.,* battery supply over a long period).

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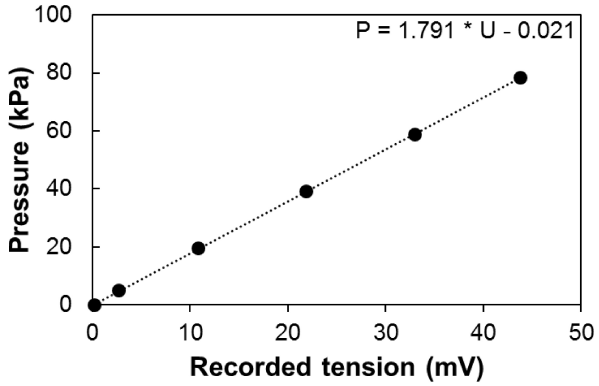

Figure 6. Calibration of the sensor. Pressure depending on the recorded tension U_{out}, with constant input tension (U_{in} = 12V).

3. The pressure is therefore equal to:

$$P = \alpha \cdot k \cdot (U_{out} - U_0)$$

where, P is the pressure in kPa, U_{out} is the output signal in mV, U_0 is the offset in mV, k the calibration coefficient of the sensor in kPa mV^{-1} and α, the ratio between U_{cal} (the input tension during calibration) and U_{in}.

4. Correcting the effect of temperature on the output signal.
   U_{out} is affected by the temperature resulting in 3.5 kPa variation in apparent pressure over the 10-20 °C range in both a closed sensor (orange line Figure 7) and a sensor connected to an excised plant *i.e.,* detached from the root system (green line Figure 7).
   The change in apparent pressure depending on the temperature is used to correct the apparent pressure (Figure 8): sensor connected to the excised plant ($U_0 = 0.530 \cdot \theta - 9.60$) or closed sensor ($U_0 = 0.482 \cdot \theta - 11.12$).
   However, the discrepancy between apparent and corrected pressure is relatively small compared to the pressure generated by the plant (50 kPa or more in intact plant, vs. *ca.* 3.5 kPa in control sensors, in this example).

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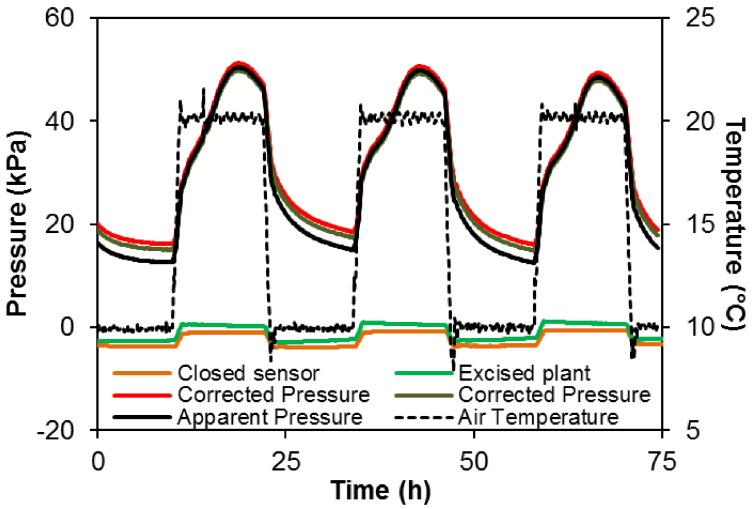

Figure 7. Variation in temperature (+ 20/10 °C; dotted black line) and the effect on the recorded signal from a pressure sensor alone (orange line) or inserted in a cut grapevine plant (without root system, green line). The change in the apparent pressure (*ca.* 0.35 kPa $°C^{-1}$) has to be taken into account in data analysis. The solid black line represents the apparent pressure recorded in an intact grapevine and red and brown lines the corrected pressure according to the correction using sensor alone or inserted in a cut plant, respectively.

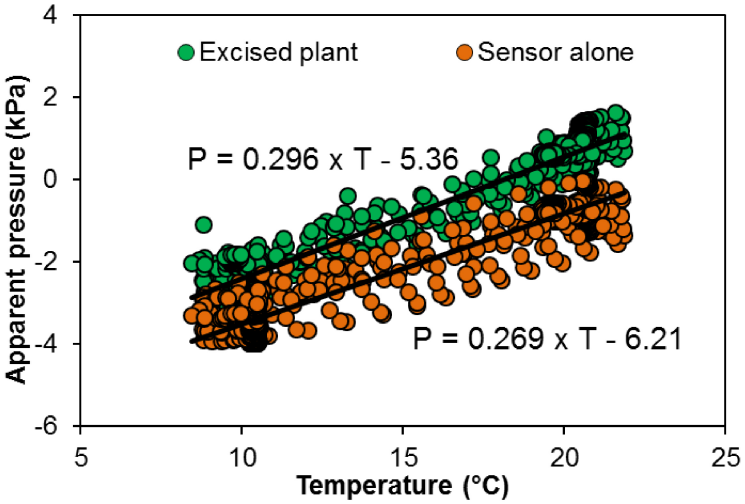

Figure 8. Apparent pressure depending on air temperature in a closed pressure sensor (orange dots) or inserted in a cut grapevine plant (without root system, green dots)

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