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TECHNICAL REPORT

A technique for measuring xylem hydraulic conductance under high negative pressures

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ABSTRACT

A technique for measuring hydraulic conductances of excised xylem segments exposed to high negative pressures is described. A centrifugal force is used to generate negative pressures (*P*) in the sample and to create a positive hydrostatic pressure difference (Δ*P*) between its two ends. Δ*P* forces water through the sample at a flow rate (*F*) determined optically during centrifugation. The sample hydraulic conductance *k* is derived from *F* and Δ*P*. The sample vulnerability curve is given by the dependence of *k* on *P*. Results for *Cedrus atlantica* Manetti and *Laurus nobilis* L. shoots are given. The technique is appropriate for the analysis of xylem refilling under negative pressure.

Key-words: *Laurus nobilis* L.; cavitation; centrifugation; embolism; water relations.

INTRODUCTION

When xylem sap is exposed to large negative pressures (*P*), cavitation can occur and air embolism can form. The dependence of xylem embolism on *P* is called a vulnerability curve (Tyree & Sperry 1989). Vulnerability curves have already been established for many species and plant parts (see Cruiziat, Cochard & Améglio 2002 for a recent review) and they have provided new and important insights into the understanding of plant water relations. Indeed, the risk of xylem cavitation sets a functional limit that can constrain leaf water loss (Sperry 2000; Cochard *et al*. 2002). Therefore, there is an increasing need for reliable and rapid techniques for establishing vulnerability curves.

Several techniques have been proposed, which differ in the ways in which *P* and/or embolism are assessed (Tyree & Dixon 1983; Sperry, Donnelly & Tyree 1988; Cochard, Cruiziat & Tyree 1992). The most commonly used method is based on a hydraulic determination of the amount of embolism (Sperry *et al*. 1988). The technique quantifies the reduction in xylem conductance due to embolism in excised plant parts. This technique has recently been criticized because xylem pressure has to be released to the atmospheric value during measurement and embolized vessels may therefore refill (Canny 1998). The extent of this problem is debatable (Stiller & Sperry 1999) and the problem is probably minor for most species (Cochard *et al*. 2000; Cochard, Améglio & Cruiziat 2001a, b). However, some species seem to present evidence of xylem refilling, even at negative *P*-values (Holbrook & Zwieniecki 1999; Tyree *et al*. 1999). For such species, the current hydraulic technique is likely to underestimate the actual amount of embolism *in planta*. Our objective was to develop a method for measuring the degree of embolism on excised samples still exposed to their negative *P*-values before excision.

Centrifugal forces have recently been employed to decrease xylem pressure and generate vulnerability curves (Pockman, Sperry & Oleary 1995; Alder *et al*. 1997). In these studies, vulnerability curves were constructed by exposing different samples to different centrifugal forces or by repetitively measuring the hydraulic conductance of the same sample when exposed to increasing forces. However, sample conductance was still measured at zero pressure, which does not rescind the criticism noted above. In a recent work (Cochard *et al*. 2000), we described a method which uses the centrifugal force to measure xylem conductance on samples exposed to negative pressures. However, in this study, it was necessary to stop the centrifuge and release the xylem pressure repetitively to measure the water flow through the sample gravimetrically. In the present article, we present an alternative procedure enabling xylem conductance measurements during centrifugation with continuous exposure to negative pressures.

MATERIALS AND METHODS

Theory

The principle of the technique is to spin a xylem segment by its centre and use the centrifugal force to both generate negative pressures (*P*, MPa) in the sample and a positive hydrostatic pressure difference (Δ*P*, MPa) across the sample. If the ends of the sample are immersed in water, the pressure difference creates a water volume flow rate *F* (mmol s^{-1}) through the sample enabling the determination of *k*, the sample hydraulic conductance as:

$$k = F/\Delta P \quad (1)$$

The dependence of *k* on *P* is then used to construct the sample vulnerability curve. The pressure difference across the sample is generated by placing the water levels in the res-

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ervoirs receiving each sample end at different distances from the axis of rotation (Fig. 1). ΔP is then computed as:

$$\Delta P = 0{\cdot}5\rho\omega^2\left[R^2 - (R-r)^2\right] \quad (2)$$

where ρ is the density of water (1000 kg m^{-3}), ω the angular velocity (rad s^{-1}), R the distance (m) from the rotational axis to the distal (downstream) reservoir, and r the difference of water level (m) between the two reservoirs (counted positive). The minimum (most negative) xylem pressure in the middle of the sample is given by:

$$P = -0{\cdot}25\rho\omega^2\left[R^2 + (R-r)^2\right] \quad (3)$$

A hole in the downstream reservoir maintained its level (and R) constant throughout the experiment. As water flows through the sample, the level in the upstream reservoir decreases at a rate equal to dr/dt. F can then be determined as:

$$F = s\mathrm{d}r/\mathrm{d}t \quad (4)$$

where s is the cross-sectional area of water in the reservoir. If the end of the sample end immersed in the upstream reservoir has a constant cross section, then s is a constant.

As it is difficult to determined F instantaneously according to Eqn 4, F was determined over a time interval as:

$$F^* = s(r_1 - r_2)/(t_2 - t_1) \quad (5)$$

where r_1 and r_2 are, respectively, the water levels in the upstream reservoir at times t_1 and t_2. The mean pressure difference between t_1 and t_2 is obtained by integrating Eqn 2 between r_1 and r_2:

$$\Delta P^* = 1/6\,\rho\omega^2\left[3R^2(r_1 - r_2) + (R-r_1)^3 - (R-r_2)^3\right]/(r_1 - r_2) \quad (6)$$

k is then computed as:

$$K = F^*/\Delta P^* \quad (7)$$

Experimental set up

Samples were held in a 28 cm long aluminium bar, firmly screwed on the rotor of a centrifuge (Model C 4·11; Jouan, Saint Nazaire, France). The original cover of the centrifuge was removed and replaced by a transparent 1·5-cm-thick Plexiglas top. The ends of the sample were placed in 4 cm^3 polystyrene vials filled with water at the beginning of the experiment. A small hole was made, respectively, at 1 and 2·2 cm from the bottom of the downstream and upstream reservoirs. R was then equal to 13 cm and r varied between 0 and 1·2 cm. It was necessary to refill the upstream reservoir periodically during centrifugation. This was technically rather difficult. One solution was to glue a tiny piece of plastic tubing on the aluminium bar with one end in the upstream vial. The other end protruded out of the centrifuge through a hole in the cover. The hole was centred on the rotational axis of the centrifuge. A second smaller plastic capillary was mounted on the needle of a water-filled syringe and inserted a few centimetres into the plastic tubing. When the syringe was pressed, water flowed through the tubing to the vial.

The major difficulty we faced was to measure the water

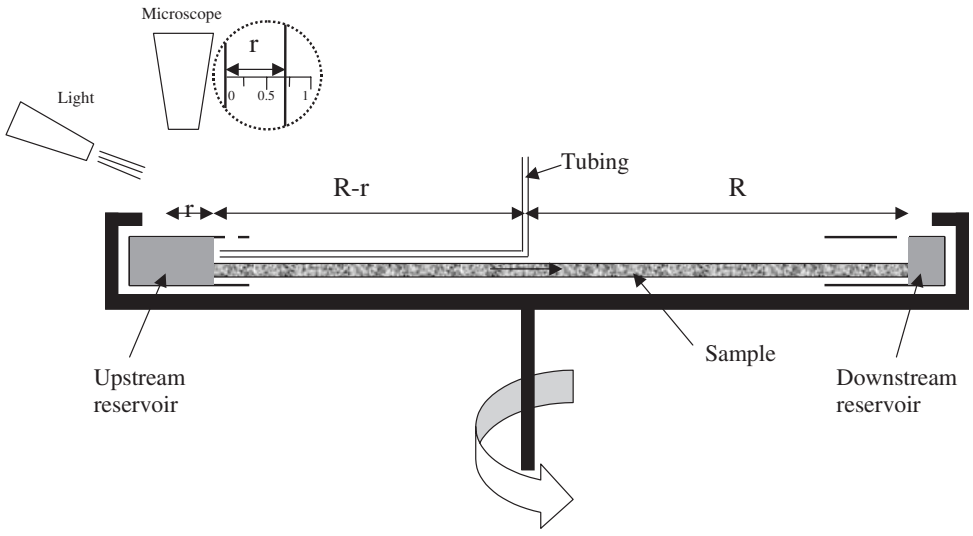

Figure 1. Schematic drawing of the experimental set up designed for continuous measurement of hydraulic conductance of xylem segments exposed to negative centrifugal pressures. The sample is centred on the axis of a centrifuge with its ends immersed in water contained into two plastic reservoirs. The maximum water level in each reservoir is determined by the position of a hole in the wall. Water can be forced through a tubing to refill the upstream reservoir. The hole in the upstream reservoir is located 1·2 cm closer to the axis of rotation, therefore creating a positive pressure difference and a water flow (F) from the upstream and downstream reservoirs through the sample. The water level (R) in the downstream reservoir is constant because water is expunged through the hole. The water level in the upstream reservoir ($R - r$) decreases over time proportionally to F. The value of r is determined optically during centrifugation by measuring the distance between the air–water meniscus in the reservoirs.

level in the reservoirs during centrifugation. The level was measured optically with a dissecting microscope. Provided that a light was directed toward the reservoirs, it was possible to observe the air–water meniscus in each reservoir and measure the water level difference (r) with a micrometer. The light was delivered by a fibre-optic illuminator – the reason why we had to place a transparent top on the centrifuge. The meniscus were best seen when the centrifuge was in a dim room. It is important to note that centrifuges can be very hazardous. The placing of an aluminium bar on a rotor and replacing the original cover of a centrifuge should only be done after careful consideration of safety and other matters. The author will send pictures of the device on request.

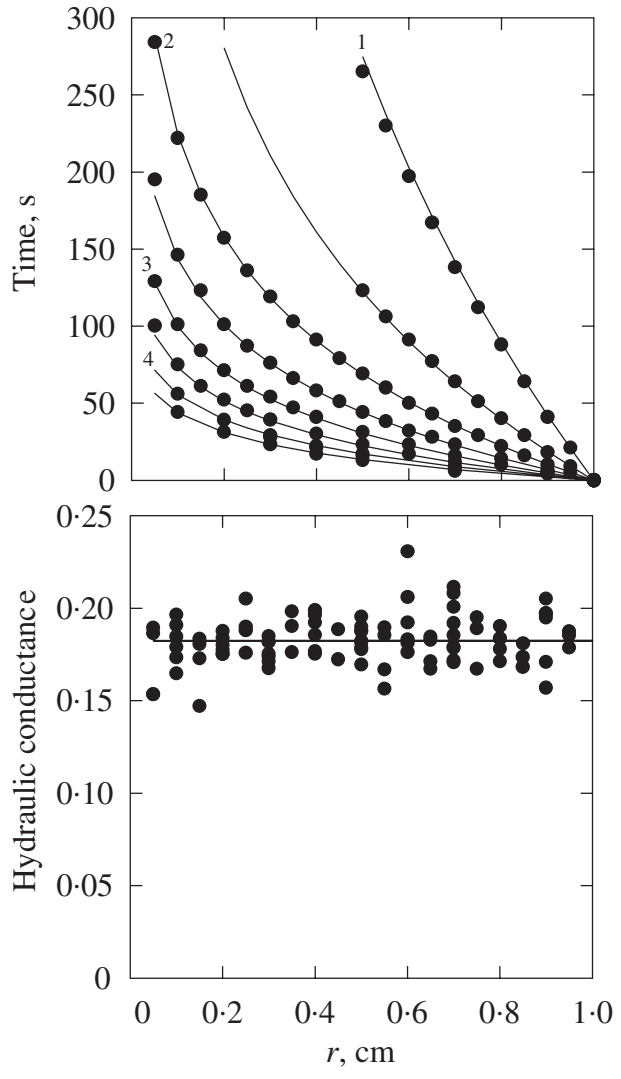

Figure 2. Upper panel: typical changes over time in water level in the upstream reservoir (r) for a *Cedrus* sample exposed to different angular velocities. The numbers on the graph represent the angular velocity in 10^3 r.p.m. The circles represent the experimental data and the lines are computations assuming a constant xylem conductance. Lower panel: Hydraulic conductance values derived from the data in the upper panel. The strait horizontal line corresponds to the constant conductance value.

The procedure for measuring k is now described. The rotational velocity (ω) was adjusted to a desired value. The distant meniscus (downstream reservoir) was positioned on the zero graduation. Water was forced through the plastic tubing to refill the upstream reservoir. The distance r was then equal to 1·2 cm and started to decrease because water flows to the downstream reservoir through the sample. When the proximal meniscus (upstream reservoir) was positioned on the 1·0 graduation, a chronometer was started and the time t was noted whenever r had decreased by 0·05 cm. F^* was then computed according to Eqn 5. We did not measure s in our experiments as only relative water flows are needed to construct a vulnerability curve. The values of ΔP^* and k were eventually computed according to Eqns 6 and 7, respectively. At the end of the procedure the upstream reservoir was refilled with water and the measurement repeated with the same or a different rotational speed. Therefore, it was not necessary to stop the centrifuge during the whole course of a vulnerability curve determination. Software was developed on a PC to measure t and solve the different equations.

Test experiments

Test experiments were conducted on *Cedrus atlantica* Manetti and *Laurus nobilis* L. shoots. *Cedrus* was selected because it is known, from previous studies (Cochard 1992), that cavitation develops in this species only for values of P below –4 MPa. Therefore, it was reasonable to assume that k was constant for P between 0 and –2 MPa, which was a necessary condition to validate some aspects of the technique. The reason for choosing *Laurus nobilis* is different. It has been suggested (Tyree *et al.* 1999) that rapid xylem refilling might occur in this species and that the hydraulic methods developed so far might not be appropriate. *Laurus* was therefore the kind of species that may best benefit from the development of such a technique. Branches were cut from field-grown trees during summer 2001. Unramified, 28 cm long, 0·5 cm diameter shoots were excised under water. The samples were manipulated and installed on the centrifuge with their ends under water to avoid air entry into the xylem.

RESULTS AND DISCUSSION

Figures 2–4 show typical results obtained with one *Cedrus* shoot. The primary data of the technique is the time dependence of the difference (r) between the water levels in the two reservoirs (Fig. 2 upper panel, circles). As expected, for a given rotation speed (ω), the time dependence was hyperbolic because ΔP decreased to zero with r. Assuming k is a constant, we combined Eqns 5, 6 and 7 and computed t for different values of ω (different lines on Fig. 2, upper panel). The data closely matched the predictions, therefore validat-

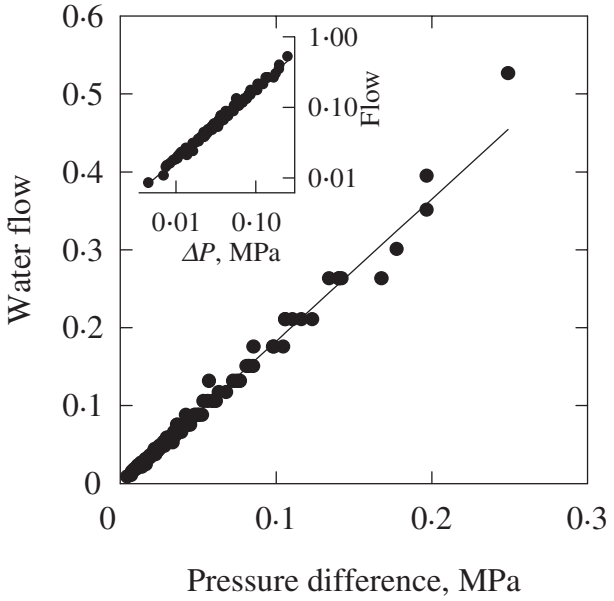

Figure 3. Water flow through a *Cedrus* sample versus pressure difference across the sample. The circles represent the experimental data and the line is a computation assuming a constant xylem conductance. The insert shows the same graph on log–log scales.

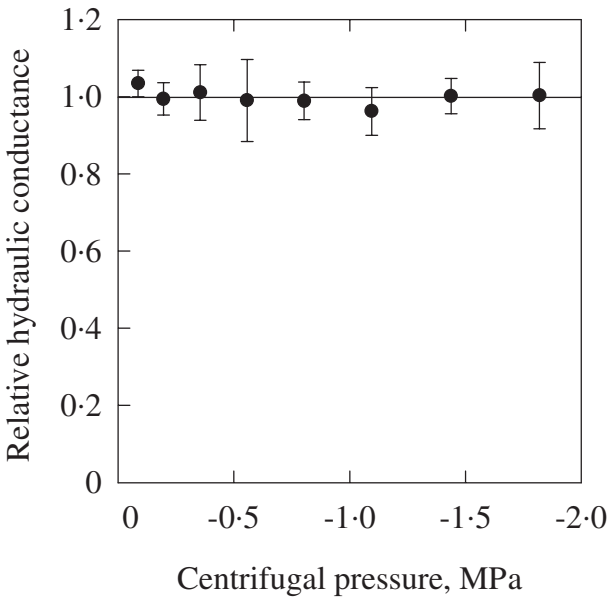

Figure 4. Relative change in xylem hydraulic conductance of a *Cedrus* sample exposed to different centrifugal pressures. Conductances were normalized by their average value. The error bars represent ±1 SE.

ing the above equations. The data on Fig. 2 (lower panel) confirmed that assuming k constant was a reasonable assumption for this trial. Figure 3 shows the relationship between the water flow through the sample (Eqn 5) and the pressure difference driving the flow (Eqn 6). Whatever the rotational speed and whatever the pressure difference, ΔP^* and F^* varied linearly and so k, the slope of the relation in Fig. 3, was thus independent of both F^* and ΔP^*. This suggests that rather high pressure differences (up to 0·3 MPa) and correspondingly high water flows do not modify the xylem conductance. The value of k varied insignificantly with P for pressures in the range of 0 to –2 MPa (Fig. 4) which confirmed our hypothesis that no embolism forms at such pressures in *Cedrus*.

The technique we have developed satisfies our main objective because it enables continuous xylem conductance measurement of samples exposed to negative xylem pressures. We have applied the technique to *Laurus* shoots, a species that is likely to exhibit rapid xylem refilling when pressure is released. Figure 5 shows the time course of an experiment conducted on a *Laurus* shoot. The centrifugal pressure was first decreased to –3·1 MPa, then increased to –0·8 MPa and maintained at this value for about 4 h. Sample conductance remained maximum until P reached –2 MPa and was drastically reduced below (Fig. 5, insert). After 4 h at –0·8 MPa, no evidence for a conductance recovery was noticed. Our objective was not to solve the issue of xylem refilling in Laurel, which would require a much more comprehensive study, but simply to show the usefulness of our technique for solving such issues. The failure to observe repair under our experimental conditions does not necessarily imply that it would not occur in an intact plant. With this technique, it is possible to reproduce experimentally the *in planta* xylem water status and provoke xylem cavitation. The water status can then be manipulated at will, which would help to define the conditions under which xylem refilling can occur in such species.

The technique could also potentially be employed to measure xylem conductances of porous or irregularly

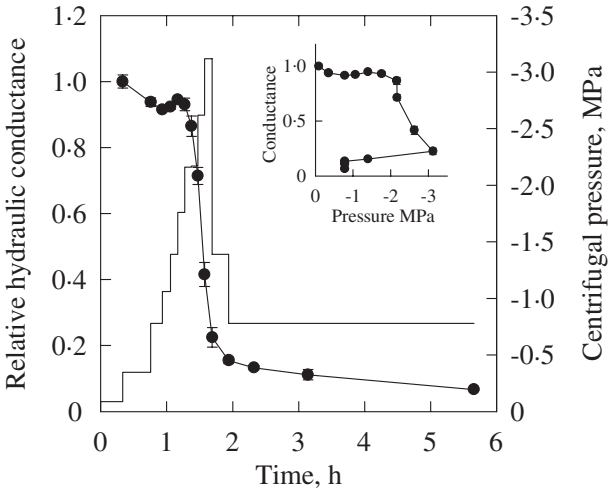

Figure 5. Time course of hydraulic conductance (circles) and negative centrifugal pressure (line) for a *Laurus* sample. The dependence of conductance on pressure is shown in the insert. The error bars represent ±1 SE.

shaped plant parts. Indeed, the ends are simply immersed in water and no seals are necessary. Furthermore, the xylem pressure in the apoplasmic compartment is always negative in the sample, which prevents artifacts caused by intercellular air spaces refilling during conductance measurement (Martre, Cochard & Durand 2001).

The technique provides an alternative method for establishing xylem vulnerability curves. It is, comparatively, rather rapid but would probably be difficult to use with large samples. It is not appropriate to directly determine the native levels of embolism. However, it is still possible to measure the native k as described in this paper, apply the conventional method to refill the xylem conduits, and measure a new value of k.

In conclusion, the technique we have developed enables continuous and prolonged measurements of xylem hydraulic conductance of excised plant parts while exposed to negative pressures. The technique can be employed to generate cavitation and construct vulnerability curves. *In planta* xylem pressures can be reproduced, which will help to identify the mechanisms of xylem refilling under negative pressure that have been hypothesized for several species.

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