Cavitation in plants at low temperature: is sap transport limited by the tensile strength of water as expected from Briggs' Z-tube experiment?

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Summary

• Xylem cavitation in plants is thought to be caused by a loss of adhesion at the conduit wall surface because a rupture in the body of the water column was implicitly ruled out by an experiment by Lyman J. Briggs with Z-tube capillaries. However, Briggs reported a drastic increase in cavitation pressure of water below 5°C which, if it were also true in xylem conduits, would suggest that water transport in plants could be limited by water cohesion at low temperature.

• In this study we have repeated Briggs' experiment using stem segments. Xylem vulnerability curves were obtained with a centrifuge technique at 1, 25 and 50°C on yew (*Taxus baccata*).

• Contrary to Briggs' finding, vulnerability to cavitation, measured as per cent loss conductance, did not increase sharply at 1°C and was even less than at 25°C and 50°C. Moreover, the onset of cavitation in yew at 1°C was measured at a much more negative pressure than Briggs' value.

• This points out an artefact in Brigg's experiment at low temperature possibly related to imperfections in the tube walls which act as cavitation nuclei.

Key words: embolism, temperature, tensile strength, water, xylem.

Introduction

Long-distance xylem sap transport occurs under large negative pressures in plants. Since sap is in a metastable state, cavitation is possible but its occurrence would disrupt water flow and hence threaten plant survival. Two distinct processes can lead to cavitation: a rupture in the body of the water column by loss of cohesion between water molecules (volume cavitation or homogeneous nucleation) or a rupture at the wall interface by loss of adhesion of water to walls (surface cavitation or heterogeneous nucleation) (Pickard, 1981). Cavitation has been well documented for plants and it is now largely accepted that volume cavitation is very unlikely, whereas air can be aspirated through xylem walls to nucleate cavitation (Sperry & Tyree, 1988). Indeed, homogeneous cavitation in plants was implicitly ruled out by an experiment by Briggs

(1950) and cited in many text books (Tyree & Zimmermann, 2002). By centrifugation of Z-shaped Pyrex glass capillaries, Briggs exposed liquid water to large negative pressures, and measured its tensile strength at which cavitation occurred ($-P_{cav}$). P_{cav} is the pressure (negative) at which a water column in a tube (e.g. glass or xylem conduit) breaks and is often referred to as the cavitation pressure. Hence the tensile force required to break a water column is $-P_{cav}$ in keeping with the notion that tension is minus pressure. Above 10°C, Briggs measured P_{cav} values below −22 MPa, far more negative than the most negative xylem pressure ever recorded for plants (*c.* −12 MPa, Sperry & Hacke, 2002). However, Briggs also noted a striking tenfold increase in P_{cav} between 0 and 5°C. Near 0°C, P_{cav} values as high as −2 MPa were measured (Fig. 1), a value typically prevailing in xylem sap of evergreen trees during winter time (Mayr *et al.*, 2003). Reid (1976), based on
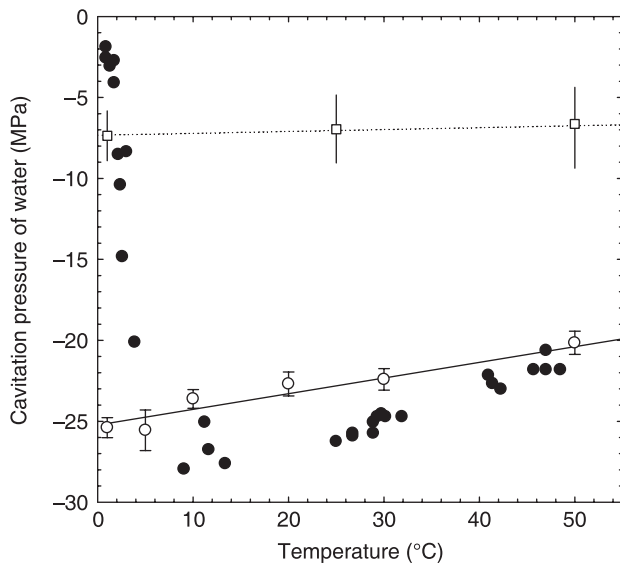
Fig. 1 Temperature dependence of the cavitation pressure (P_{cav} = –tensile strength) of water according to Briggs' Z-tube experiment (filled symbols, after Briggs, 1950). Note the very sharp change in P_{cav} for temperatures below 5°C. The open squares and the horizontal dashed line represent the xylem pressure provoking 50% loss of conductance (P_{50}) measured in this study for *Taxus baccata* at 1, 25 and 50°C. Vertical lines represent the variation of P between P_{10} and P_{90}. For temperatures above 5°C, P_{50} is fivefold less negative than the P_{cav} of water. According to Briggs' data, below 5°C, vulnerability to cavitation in *Taxus* should have increased because P_{50} should always remain less negative than P_{cav} of water. Our experiment does not support this hypothesis, which is consistent with more recent determination of tensile strength of water at low temperature in which the negative pressure develops away from any wall (open symbols, after Herbert & Caupin, 2005).

Briggs' results, pointed out that 'at temperatures below 5°C, water apparently loses much of its ability to support tensile stress' and concluded that 'Clearly sap cannot flow at temperatures near freezing, and this fact may be important in the study of plants that must survive in a freeze–thaw environment'. If Briggs' experiment applies to woody samples then we would conclude that tensile strength of water is the functional limitation for water transport in plants at low temperature.

The objective of this study was to repeat Briggs' experiment with wood samples at low temperature. Interestingly, this experiment has not been done before. We chose a conifer (*Taxus baccata*) showing no cavitation below –5 MPa at 20°C (Cochard *et al.*, 2005), which is much below the P_{cav} value for water at 1°C as reported by Briggs (1950). Samples were spun in a centrifuge at different temperatures (1°C, 25°C and 50°C) and cavitation was determined by measuring changes in sample hydraulic conductance. A sharp increase of sample vulnerability to cavitation at 1°C would demonstrate that tensile strength limits sap transport at low temperature in plants.

Materials and Methods

Experiments were carried out on shoots of a yew tree (*T. baccata* L.) growing on a dry site near the INRA campus in Clermont-Ferrand, central France. Shoots were cut from the south-exposed part of the tree to minimize possible variability between samples. Shoots were defoliated, enclosed in a plastic bag to prevent dehydration and stored at 5°C until experimentation (within 24 h). In the laboratory, the bark was removed and a 28-cm long segment was cut from each shoot under tap water. The segments were centred on an annual growth unit and their wood diameters averaged 6 mm (SD = 1.2). The segment was carefully rinsed with simply distilled water and installed in a special rotor of a Cavitron, a modified centrifuge designed for measuring xylem vulnerability to cavitation. A detailed description of this centrifuge technique can be found in Cochard (2002) and Cochard *et al.* (2005). Briefly, the centrifugal force is used to lower xylem pressure P (MPa) in the middle of the sample according to:

$$P = -0.5\rho\omega^2R^2 \qquad \text{Eqn 1}$$

(ω is the angular velocity of the centrifuge (rad s^{-1}); R is the radius of the sample exposed to negative pressure (0.13 m); and ρ is water density (999.9, 998.3 and 988.0 kg m^{-3}, respectively) at 1, 20 and 50°C).

The sample ends were inserted in two plastic containers filled with *c.* 1 cm of distilled water. The water level in one container was maintained at 1 cm whereas the level in the second container could be adjusted between 1 cm and 1.5 cm during centrifugation. The difference in water level (r, m) creates a pressure drop (dP, MPa) across the sample and hence a water flow, F, through the sample that is proportional to sample hydraulic conductance, K, according to:

$$dP = 0.5\rho\omega^2[R^2 - (R - r)^2] \qquad \text{Eqn 2}$$

$$K = F/dP \qquad \text{Eqn 3}$$

F was determined optically with a microscope by measuring the decrease in water level per unit of time (s) in the upstream container:

$$F = Dr/dt \qquad \text{Eqn 4}$$

Therefore, the technique enables the determination of sample hydraulic conductance K during centrifugation and the cavitation-induced variation of K with decreasing xylem negative pressure P. The relative dependence of K with P represents the vulnerability curve of the segment.

Vulnerability curves for four different stem segments were obtained for each of three temperatures: 1°C, 25°C and 50°C. Air temperature inside the centrifuge was controlled with a thermostatically controlled bath and measured with a

thermocouple. For experiments at 1°C the whole setup was installed in a large cold chamber regulated at 5°C. At the onset of each experiment, the minimum sample xylem pressure was adjusted to −0.5 MPa and the temperature inside the centrifuge set to the predefined value (< 0.5°C, 25°C or 50°C). The sample was then at the same temperature as the air in the chamber. Sample initial conductance, K_{init}, was then determined. The angular velocity was then rapidly (within 1 min) increased in order to obtain xylem pressures in the range of −1 to −8 MPa. The xylem pressure was maintained for about 1 min at the target pressure and then rapidly (within 1 min) returned to −0.5 MPa. Once the temperature was returned to the preset value, sample conductance K was determined again. Per cent loss conductance (PLC) was then computed as:

$$PLC = 100(1 − K/K_{init}) \qquad \text{Eqn 5}$$

The procedure was then repeated with a more negative value of the target pressure in order to construct the whole vulnerability curve.

It was necessary to expose samples to large negative pressure for only a short period to prevent a substantial temperature rise in the centrifuge. The actual air temperature increase depended on the angular velocity, that is, on the negative pressure. For example, at −5 MPa air temperature increased in the range of 1–2°C. At −7 MPa the increase reached 5°C. However, the rotor was made of a 3-cm thick aluminium disk and thus had a large thermal inertia; therefore, the actual sample temperature increase was probably much less than the air temperature increase we measured.

Cavitation pressure in a single glass tube is determined by a single value (that vary between successive trials). Cavitation is a more progressive process in wood because it is composed of thousands of conduits with different sizes and anatomies. If cavitation is caused by a decrease in water tensile strength at low temperature, then we would expect to measure 100 PLC at the P_{cav} value measured by Briggs. Hence, P_{cav} should be compared with P_{100}, the xylem pressure causing 100 PLC. However, the experimental determination of P_{100} is ambiguous; therefore, we compared P_{cav} with P_{90}, P_{50} and P_{10}, the xylem pressures provoking 90, 50 and 10 PLC, respectively. If $P_{10} < P_{cav}$ then *a fortiori*, $P_{100} < P_{cav}$. P_{10}, P_{50} and P_{90} were determined by fitting logistic functions to the data (Pammenter & Vanderwilligen, 1998).

Results

At room temperature (25°C), cavitation, detected by loss of sample conductance, occurred in *Taxus baccata* only when xylem pressure was decreased below c. −5 MPa, 50% loss conductance being noted at c. $P_{50} = −7$ MPa (Fig. 2 and open squares in Fig. 1). At 50°C a slight increase in xylem vulnerability was noted ($P_{50} = −6.5$ MPa). At 1°C, xylem vulnerability was lower than at room temperature, loss of
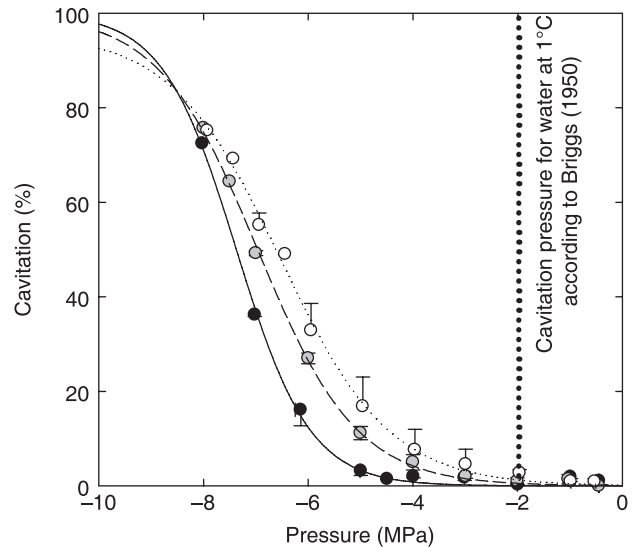

Fig. 2 Temperature dependence of xylem cavitation in *Taxus baccata*. Xylem cavitation (% loss conductance) was determined by centrifuging wood samples at three different temperatures (filled circles, 1°C; tinted circles, 25°C; open circles, 50°C). The vertical dotted line represents the cavitation pressure of water at 1°C as determined by Briggs (1950). According to this work, loss of conductance caused by cavitation in *Taxus* at 1°C should occur at −2 MPa due to a loss of water cohesion. Contrary to this expectation, vulnerability to cavitation increased with temperature from 1°C to 50°C.

conductance being noted only for xylem pressure c. −6 MPa ($P_{50} = −7.4$ MPa). The per cent loss conductance between −2 MPa and −5 MPa at 1°C was negligible and P_{10} equalled −5.8 MPa.

Discussion

According to Briggs' (1950) classical experiment, the tensile strength of water is very high at room temperature but decreases very sharply below 5°C. At 1°C, Briggs found that a water column in a Pyrex glass capillary was unable to sustain negative pressures lower than −2 MPa. The aim of the present study was to evaluate if Briggs' findings apply to water columns in wood and hence sap transport in plants. To test this hypothesis, two conditions were required. First, it was necessary to find a species showing no cavitation at room temperature below −2 MPa, otherwise it would have been difficult to distinguish cavitation caused by air seeding through xylem walls from a loss of water cohesion caused by a low water tensile strength. Since the onset of cavitation in *Taxus* at room temperature was c. −5 MPa (Cochard *et al.*, 2005), this first condition was fulfilled. The second condition was to be able to detect cavitation on refrigerated samples exposed to large negative sap pressures. The Cavitron technique (Cochard *et al.*, 2005) offers these possibilities since readings can be made as the stem samples are spun in the ultracentrifuge.

Although we noted an increase in air temperature at high rotational velocity, we are confident that samples were exposed to temperatures near 1°C in the range of 0 to −5 MPa. Therefore, our experimental setup was appropriate to test Briggs' hypothesis.

According to Briggs' results, a water column in a tube cavitates above −2 MPa at 1°C. If this applied to xylem conduits, a massive cavitation resulting in 100% loss of conductance (100 PLC) should have been detected at 1°C in yew samples exposed to xylem pressure below −2 MPa. This was clearly not the case as no more than 10 PLC was induced at −5.8 MPa. Furthermore, sample resistance to cavitation (i.e. PLC) tended to increase with decreasing temperatures. Therefore, Briggs' observations with water columns in Z-glass tubes did not apply to water columns in wood sample. To try to understand this discrepancy, it is interesting to compare different cavitation experiments. Several groups have used the centrifugal method: it was introduced by Reynolds (1882), who observed that the water column in a sealed U-tube cavitated at $P_{cav} = −0.49$ MPa at room temperature. Temperley & Chambers (1946) repeated Reynold's experiment and obtained tensions between 0 and −0.57 MPa for tap water. Briggs (1950) used boiled distilled water in Pyrex glass capillaries 0.6–0.8 mm internal diameter. He mentioned that 'scrupulous cleanliness is necessary in the measurement of negative pressure', but did not document the reproducibility of his results. Strube and Lauterborn (1970) also used the centrifugal method to study pure degassed water in quartz tubes. The tensions they found vary widely (between −0.08 and −17.5 MPa) between tubes, or even with time for a given tube. The wide range of tensions obtained in these experiments suggests that water cleanliness and wall surface plays a major role. This hypothesis was tested more recently by Smith (1991) using Z tubes.

Among all the experiments investigating the centrifugal method, only Briggs reported the dependence of cavitation pressure on temperature. Recently, Herbert & Caupin (2005) used an acoustic method to study cavitation. Thanks to a focused ultrasonic wave, they were able to investigate a small volume of degassed ultrapure water. The acoustic wave is focused in the liquid: the negative pressure develops away from any wall. Their results (shown in Fig. 1) are reproducible, and show that cavitation occurs in a narrow range of tensions. They found a temperature dependence in good agreement with Briggs' data at high temperature; however, they observed a monotonic temperature variation, which contrasts with the sharp decrease observed by Briggs at low temperature.

We should also mention experiments by Zheng *et al.* (1991) using microscopic inclusions of water in a quartz crystal. There is some uncertainty about the pressure reached in the inclusion: using an extrapolation of the equation of state for water, Zheng *et al.* estimated a tension *c.* −140 MPa at 40°C. Interestingly, no cavitation was observed at lower temperature. With this method, the water sample followed a quasi-isochoric path, and, as water possesses a line of density maxima,

the tension passes through a maximum when the temperature decreases; much greater tensions than those observed by Briggs' could be obtained at low temperature, without causing cavitation.

The comparison of these different experiments suggests that the effect of walls and the preparation of the water sample are major issues in cavitation studies. Briggs stated that he could not 'tell whether the rupture of the columns originates on the wall of the capillary (loss of adhesion) or in the body of the liquid (loss of cohesion)'. To know if the xylem walls are less favourable to cavitation than other materials, it would be interesting to compare cavitation thresholds of water prepared under the same conditions in xylem segments and in glass tubes.

Xylem cavitation in yew exhibits a relatively small temperature dependence. In the range of 1–50°C, P_{50} increases by 11.5%. It is relatively difficult to identify the reason for such a dependence. Indeed, cavitation in plants is thought to be caused by air entry through holes in conduit walls (presumably at pits or cracks in the walls). Temperature is likely to have affected many parameters involved in this mechanism. Variation in water surface tension with temperature (9.1% increase between 1°C and 50°C), probably explains most of the variation in xylem vulnerability to cavitation. However, change in pit membrane porosity or change in microfibril rigidity in pit margo may also have occurred (Hacke *et al.*, 2004). Nevertheless, these variations with temperature remain small and are probably negligible under field conditions.

In conclusion, cavitation caused loss of conductance in the xylem of *Taxus* segments at pressures below −5 MPa whether measured at 1°C or 20°C. At 20°C cavitation was caused by a loss of adhesion between water and the conduit walls because loss of cohesion between water molecules occurred at pressured below −20 MPa (Briggs, 1950; Herbert & Caupin, 2005). Our results imply that cavitation also occurred by loss of water adhesion to walls at 1°C in agreement with recent estimates of water tensile strength at low temperature (Herbert & Caupin, 2005). This suggests that Briggs' results in the range from 0 to 5°C are possibly artefacts – a consequence of the failure of water adhesion to the Z-tube glass walls. Remarkably, xylem conduits in trees are thus able to maintain high cohesive forces between their walls and water molecules even at near freezing temperatures.

Acknowledgements

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