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RESEARCH PAPER

The effects of sap ionic composition on xylem vulnerability to cavitation

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Abstract

Recent evidence of ion-mediated changes in pit membrane porosity suggests that plants may modulate the hydraulic conductance of their xylem conduits. Under the current paradigm, membrane porosity also determines conduit vulnerability to water stress-induced cavitation. Therefore, the hypothesis of an ion-mediated regulation of xylem vulnerability to cavitation in trees was tested. Segments of five Angiosperm and two Gymnosperm species were infiltrated with ultra-pure deionized water as a reference fluid or with a 50 mM KCl solution. KCl had a strong impact on segment conductance with either a positive or a negative effect across species. When 1 mM $CaCl_2$ was added to the reference solution, the effect of KCl was minimized for most species. By contrast, segment vulnerability to cavitation was only slightly influenced by the presence of KCl in the solution. From this it was concluded that the mechanisms controlling pit membrane permeability to water flow and its resistance to the penetration of air bubbles are largely uncoupled, which suggests that the hypothesis of a porous structure of pit membranes should be revisited.

Key words: Cavitation, hydraulic conductance, pectin, pit membrane, xylem.

Introduction

Long-distance sap transport in plants occurs in xylem conduits having small diameters and finite lengths. Sap flows between adjacent conduits through pits that form pores in the walls. These structures provoke frictional losses and a resistance to the water flow and, therefore, induce large negative sap pressures (Lancashire and Ennos, 2002). The functional significance of tree hydraulics has become increasingly clear and experimental evidence shows that these traits may affect leaf gas exchange (Hacke and Sperry, 2001; Cochard, 2002*a*; Lemoine *et al.*, 2002), tree growth (Tyree, 2003; Daudel *et al.*, 2005; Cochard *et al.*, 2007), mortality (Brodribb and Cochard, 2009), and species distribution (Ewers *et al.*, 1997; Kursar *et al.*, 2009).

Pit membranes are modified primary cell walls made of tightly interwoven cellulose microfibrils in a matrix of hydrated hemicellulose and pectins. Pectins account for about one-third of all wall macromolecules (Jarvis, 1984; Willats *et al.*, 2001*a*), and consist of complex galacturonic acid (GalA)-rich polysaccharides. GalA can be assembled into two structural types forming the backbone of three main polysaccharide domains that have been isolated and structurally characterized. These are homogalacturonan (HG), rhamnogalacturonan (RG)-I, and RG-II.

Pectins may be regarded as immobilized anionic polyelectrolytes, and the hydration and swelling properties of such charged polymers are influenced by ionic interactions (English *et al.*, 1996; Tibbits *et al.*, 1998; Ryden *et al.*, 2000). Moreover, the hydration and swelling behaviour depends on the equilibrium between neutral carboxylic residues due to methyl esterification and exposed negative charges of dissociated carboxyls (Ryden *et al.*, 2000). An important contribution to pectin swelling and hydration

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arises as a result of the Donnan effect producing an excess of cations within the pectin network compared to the surrounding solution. At low ionic strength, hydration and swelling increase as there is a Donnan effect around the immobilized charges. At higher ionic strength, obtained with the perfusion of KCl or $CaCl_2$ solutions, the negative charges from GalA are shielded resulting in shrinking and dehydration of pectins. Polyelectrolyte swelling, arising as a result of a Donnan effect, is not the only way contributing to the swelling behaviour of pectin. Ca^{2+} cations cross-link GalA groups of antiparallel chains of pectins together, in an 'eggbox' model (Grant *et al.*, 1973). Dissociation of these calcium cross-links results in an increased swelling of pectins, and this phenomenon has been observed when the Ca^{2+}-concentration is reduced in the bathing solution (Tibbits *et al.*, 1998). Pectins capable of Ca^{2+} cross-linking are particularly common in bordered pit membranes (Chaffey *et al.*, 1997; Hafren *et al.*, 2000). Accordingly, ion-mediated variations of xylem conductance have been attributed to the hydrogel properties of pit membrane pectins (van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001; Boyce *et al.*, 2004). According to these authors, plants may modulate their xylem hydraulic resistance by adjusting their sap ionic composition.

Pit membrane properties not only influence the passage of water between conduits, they also control the passage of air from embolized conduits to adjacent ones. Indeed, cavitation in plants is thought to be caused by the rupture of air–water menisci that form in the pores of pit membranes (Sperry and Tyree, 1988). When xylem pressure drops below a threshold value determined by pore diameter (Young–Laplace equation), an air bubble is aspirated which nucleates the conduit (Cochard, 2006). Conduits with smaller porosity in their pit membrane are thought to be capable of sustaining more negative pressures before cavitation. Therefore, if sap ionic composition influences pit membrane porosity through swelling behaviour of pectins, it can be hypothesized that it may also affect xylem vulnerability to cavitation. The benefit of an increased ion-mediated xylem conductance could then be jeopardized by an increased vulnerability to cavitation. To our knowledge, this hypothesis has not been evaluated. Gascó *et al.* (2006) have nicely documented that an increased sap ionic strength lowers the effect of cavitation on loss of xylem hydraulic conductance because sap can better by-pass the embolized conduits. However, the effects of ions on xylem vulnerability to cavitation *per se* were not tested in their study.

In this study, the effects of the presence of ions in the sap on both the xylem vulnerability to cavitation and the xylem conductance of seven different tree species are reported. Shoots were perfused with deionized water or with a solution of 50 mM KCl and sample vulnerability to cavitation was determined with a centrifuge technique (Cochard *et al.*, 2005). The effect of KCl on the xylem conductance was also documented and, on different species, a recent observation of van Ieperen and van Gelder (2006) suggesting that the presence of calcium ions in the solution inhibits the ion-mediated changes in xylem conductance was repeated.

Materials and methods

Plant material

Most experiments were conducted on seven species planted in the King's Buildings campus, Edinburgh University (Edinburgh, UK). Shoots of five broadleaved species (*Salix alba* L., *Betula alba* L., *Fagus sylvatica* L., *Prunus avium* L., and *Tilia platyphyllos* Scop.) and two conifers [*Pinus sylvestris* L. and *Cedrus atlantica* (Manetti ex Endl.) Carrière] were sampled between September and November 2006. Xylem water retention curves were obtained on the same species but on trees sampled between September and November 2008 in the INRA-Crouël campus (Clermont-Ferrand, France). Shoots were collected from sun-exposed branches of mature trees. To minimize any possible intraspecific variations, all shoots were sampled on one tree for each species. Shoots were collected in the morning and analysed for ionic effects during the same day. In the laboratory, 0.28 m long samples were cut under tap water from the main shoot axis. For conifers, the bark was stripped off at each extremity. Sample diameter ranged from 5 mm to 9 mm. Broadleaved species were all of the 'diffuse porous' type, and at the end of typical experiments, it was verified that air forced at 0.1 MPa did not pass through the samples. This demonstrated that vessels were shorter than the sample length for these species.

Experimental protocols

The ionic effects on xylem conductance were determined using a combination of very low and high pressure gradients. The objective of the first experiment was to mimic the impact of sap ionic composition *in planta*, hence a pressure gradient of 14.3 kPa m^{-1} was used. The second experiment aimed at determining the effect of sap ionic composition on pit membrane flexibility in conifers, which required much higher pressure gradients (up to 5 MPa m^{-1}).

Ionic effects on xylem conductance

For each species, hydraulic conductances (K, mmol s^{-1} MPa^{-1}) were determined with a XYL'EM apparatus (Bronkhorst, Montigny-les-Cormeilles, France) on ten samples. First, the samples were perfused at 4 kPa with ultra-pure deionized water as a reference fluid (MilliQ ultra-pure water system, Millipore, France) and K_{init} determined. Then samples were perfused with the same solution for 10 min at 0.15 MPa and K determined at 4 kPa as above. The procedure was repeated until K stabilized (K_{H_2O}), typically after 1 hour. The change in sample xylem conductance (ΔK_{H_2O}) caused by perfusion with ultra-pure water was computed as:

$$\Delta K_{H_2O}\% = 100 \times \left(\frac{K_{H_2O}}{K_{init}} - 1\right) \tag{1}$$

The xylem vulnerability to cavitation was then determined for half of the samples (n=5, see below). For the other samples (n=5), the solution was replaced by an ultra-pure deionized water solution with 50 mM of KCl and sample conductance determined anew at 4 kPa. Again, the sampled were perfused with the same KCl solution at 0.15 MPa until a new stable K value was obtained (K_{KCl}). The ion-mediated change in xylem conductance was computed as:

$$\Delta K_{KCl}\% = 100 \times \left(\frac{K_{KCl}}{K_{H_2O}} - 1\right) \tag{2}$$

Sample vulnerability to cavitation was also determined for this second set of five samples, as described below.

In a second experiment (n=5), the procedure above was repeated using 1 mM $CaCl_2$ in ultra-pure deionized water as a reference fluid. One mM $CaCl_2$ was also added to the 50 mM KCl solution. Xylem vulnerability to cavitation was not determined for these samples.

Ionic effects on pit membrane flexibility

The effect of sap ionic composition on pit membrane flexibility in conifers was assessed by perfusing, under high pressure, 0.28 m long segments with deionized water or a 50 mM KCl solution. High pressure gradients displace pit torus against pit chamber walls which decreases xylem conductance. Following Hacke *et al.* (2004), it was hypothesized that the higher the pressure gradient required for pit displacement, the lower the flexibility of the fibrils in the pit margo. Experiments were conducted on *Pinus sylvestris*, and *Fagus sylvatica* was used as a control species.

Pinus segments were prepared and connected to a XYL'EM apparatus as detailed above and first perfused for 1 h at 0.1 MPa with ultra-pure deionized water (n=6) or ultra-pure deionized water with 50 mM KCl (n=7). The terminal end of each segment was inserted in a pressure chamber positioned upside down and filled with the same solution. The solution was contained in a plastic reservoir fitted directly to the pressure chamber rubber seal. With this set-up, less than 5 mm of the terminal sample end was in contact with the solution inside the chamber and no pressurized air was in contact with the sample. A water-filled tubing was fitted to the proximal sample end and connected to an analytical balance to measure the water flow through the sample. The air pressure in the chamber was first increased stepwise from atmospheric to 1.4 MPa. Between each step, the pressure was increased or decreased very slowly (typically at 5 kPa s^{-1}) in order to minimize the variation in pressure gradient along the segment. At each step, the pressure was maintained constant until the water flow through the sample stabilized. A typical experiment was as follow: the pressure in the chamber was first increased from 0 to 1.4 MPa in 0.1 or 0.2 MPa steps, then returned to atmospheric and, finally, re-increased to 1.4 MPa.

Ionic effects on xylem cavitation and loss of hydraulic conductance

Xylem loss of hydraulic conductance due to cavitation was assessed with the Cavitron technique (Cochard, 2002*b*; Cochard *et al.*, 2005), a technique derived from the centrifuge method of Alder *et al.* (1997). The technique uses the centrifugal force to increase the water tension in a xylem segment and, at the same time, measures the decrease of its hydraulic conductance. The curve of percentage loss of xylem conductance (*PLC*) versus xylem water tension represents the sample vulnerability to cavitation. Vulnerability curves were determined for five different samples for each treatment. Samples were perfused with the same fluid as the one used to measure stem conductance, i.e. ultra-pure deionized water or ultra-pure deionized water with 50 mM KCl.

Xylem pressure (P) was first set to a reference pressure (–1 MPa) and the sample maximal conductance (K_{max}) was determined. The xylem pressure was then set to a more negative pressure and subsequently returned to the reference pressure to determine the new sample conductance K. The sample percentage loss of conductance (PLC) was then computed as

$$\mathrm{PLC} = 100 \times (1 - K/K_{\max}) \tag{3}$$

The procedure was repeated for more negative pressures (with –0.125 to –0.5 MPa step increments depending on species vulnerability) until *PLC* reached at least 95%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution). Following Pammenter and Van der Willigen (1998), a sigmoid function was fitted to each curve:

$$\mathrm{PLC} = 100/[1 + \exp(s(P - P_{50})/25)] \tag{4}$$

where P_{50} is the pressure causing 50% loss of conductance (*PLC*) and s is a slope parameter. P_{50} and s values were averaged for each treatment and t tests were used to compare treatments.

As demonstrated by Gasco *et al.* (2006), the analysis of these vulnerability curves is complicated by the fact that the presence of ions in the solution may also alter radial hydraulic conductances and hence the effect of cavitation on the loss of hydraulic conductance. To detect the effect of sap ionic concentration on the process of cavitation *per se*, xylem water retention curves were constructed, i.e. the relative variation of xylem water content with xylem pressure (Tyree and Yang, 1990). Xylem segments were prepared as described above with the difference that the bark was entirely removed to lower branch symplasmic water content. Control samples were perfused for 1 h with ultra-pure water at 0.15 MPa. Samples treated with KCl were subsequently perfused for 1 h with a 50 mM solution at 0.15 MPa. The segments were then installed in a Cavitron with the two ends immersed for 1 cm in water contained in two intact (no hole) plastic reservoirs. The reservoirs were filled with ultra-pure or 50 mM KCl water solutions according to the different treatments. The rotational velocity of the centrifuge was increased stepwise which released water from the segment and increased the water level in each reservoir. The equilibrium was obtained typically in less than 2 min and the water levels in the two reservoirs tended to equilibrate rapidly, except at high velocity, presumably because sample conductance was reduced to zero. The water levels in the two reservoirs were then averaged. Retentions curves were constructed with three segments for each species and treatments.

The retention curves were typically biphasic (see Results). A variable amount of water was first released at relatively high pressures, possibly representing water stored by capillarity in the xylem apoplast, or water extracted from the wood symplast. For most species a dramatic increase in water release was noted when xylem pressure was further decreased. This threshold pressure was well correlated to the critical point of loss of conductance measured with the previous technique. This suggests that water was released by xylem cavitation beyond this pressure. To characterize more precisely the water retention curve due to xylem cavitation, the values were corrected, taking into account only the water released beyond this point of cavitation. This enabled the calculation of the pressure provoking 50% of water release (P'_{50}, MPa) by fitting a sigmoid function to each curve as described above.

Results

Ionic effects on xylem conductance

The experimental set-up in this work was similar to the set-up used by van Ieperen and van Gelder (2006), i.e. samples were first perfused with a reference fluid (deionized ultra-pure water with or without 1 mM $CaCl_2$) and subsequently perfused with the same reference solution with 50 mM KCl. Xylem conductance was measured with a low pressure head (4 kPa) after repeated high-pressure flushes (0.15 MPa). Figure 1 shows the time-courses for three species, while the mean values at steady-states for all species are given in Figs 2 and 3. For all species, sample hydraulic conductance (K) first increased after the first high-pressure perfusion with the reference fluids (Fig. 1). This variation was attributed to the resorption of native embolism and was not taken into account to compute the effect of ultra-pure water on K (equation 1). For *Fagus*, K increased moderately but significantly after each perfusion with ultra-pure water (Fig. 2, upper panel). For *Betula* and *Prunus*, the variation was insignificant. For the remaining four species, a substantial and significant K decrease was measured. The addition of 1 mM $CaCl_2$ in the reference solution significantly reduced the variation in K for *Cedrus* and *Tilia* but had no effect for *Betula*, *Salix*, *Fagus*, and *Prunus* (Fig. 2,

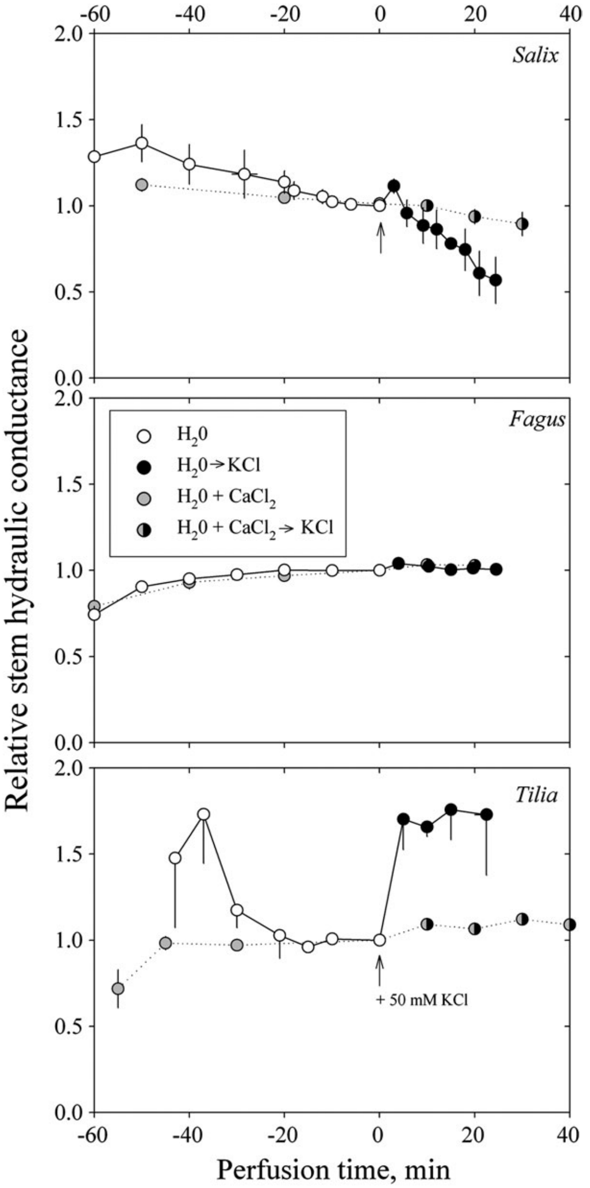

Fig. 1. Time-courses of relative stem hydraulic conductance perfused with different solutions for three tree species. Segments were first perfused with deionized water (open circles) or deionized water with 1 mM $CaCl_2$ (closed circles) as a reference fluid. At time t=0 (arrow), 50 mM KCl was added to the reference fluid. Error bars represent 1 SE (n=5).

lower panel). For *Pinus*, $CaCl_2$ significantly accentuated the decrease in sample conductance due to the ultra-pure water.

The second phase of the experiment consisted in perfusing the same samples with a 50 mM KCl solution in ultra-pure water. Across species, KCl effects on K were contrasted (Fig. 1; Fig. 3, upper panel). For *Betula*, the effect was insignificant, but for *Fagus*, *Prunus*, and *Tilia*, KCl significantly increased K. Surprisingly, a significant and substantial decrease in sample conductance was measured for *Pinus*, *Cedrus*, and *Salix*. These variations were significantly and strongly inhibited by the presence of Ca^{2+} in the solution for *Salix*, *Cedrus*, *Prunus*, and *Tilia* (Fig. 3, lower panel).

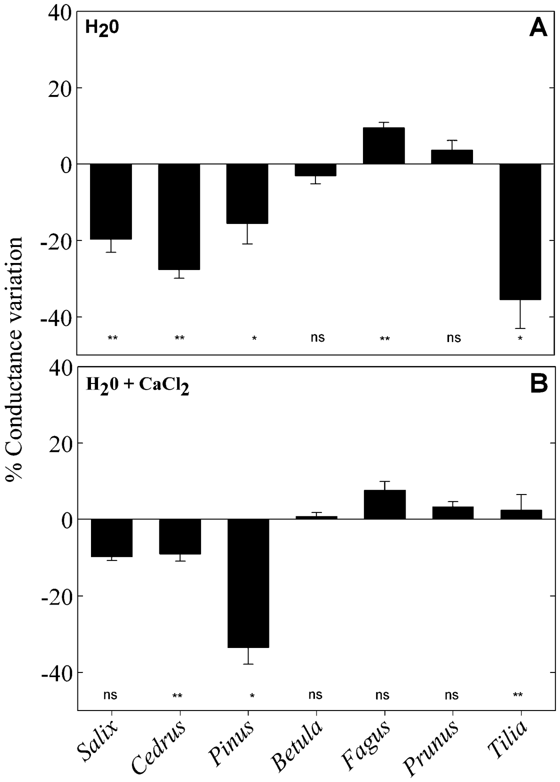

Fig. 2. Relative variation of xylem conductance for control samples perfused with deionized water (upper panel) or with 1 mM $CaCl_2$ solution (lower panel). A positive variation signifies that sample conductance has increased above its initial value. Error bars represent 1 SE (n=10).

Ionic effects on xylem loss of conductance

Figure 4 gives, for each species, the vulnerability curves obtained for samples perfused with ultra-pure water or with a 50 mM KCl solution. Overall, KCl had a small effect on xylem vulnerability. Absolute differences in P_{50} values were less than 0.3 MPa (Fig. 6, upper panel), a less than 6% relative variation. However, KCl significantly increased the xylem vulnerability to cavitation in *Fagus* and *Prunus* (i.e. P_{50} values were less negative in presence of K^+ cations), but the opposite effect was measured for *Cedrus*. Differences were not significant for the remaining species.

Ionic effects on xylem water retention

Figure 5 shows the raw and corrected water retentions curves of samples initially perfused with water or water plus KCl. KCl had a very small effect on the retention curves, the effect on P'_{50} being significant only for two species (Fig. 6, lower panel). A close correlation was found between P_{50} and P'_{50} values (r^2=0.90, P <0.05).

Ionic effects on pit membrane flexibility

Typical high pressure–flow relationships are shown for *Pinus* and *Fagus* samples in Fig. 7. During a first phase of the experiment, fluid pressure was increased from atmospheric to 1.4 MPa. For *Pinus*, fluid flow through the sample first increased linearly with fluid pressure, indicating a nearly constant xylem conductance (K_0). Above a threshold pressure, K sharply decreased to less than 20% of its initial value (K_1). This sudden decrease in conductance is attributed to pit aspiration (Bolton and Petty, 1978; Sperry and Tyree, 1990; Hacke *et al.*, 2004). The P_{50} pressure P_1 provoking 50% reduction in sample conductance was determined graphically. Fluid pressure was then released to atmospheric. K remained equal to K_1 but eventually returned to K_0 at a P_{50} pressure P_2 significantly lower than P_1 (Fig. 8). When P was increased again, a similar pattern was observed, but the conductance drop occurred at P_3 pressure intermediate between P_1 and P_2 and significantly different.

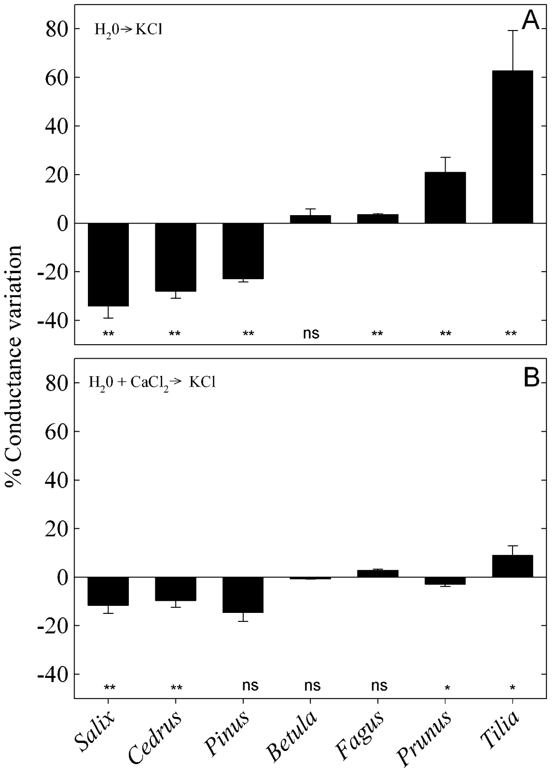

Fig. 3. Relative variation of xylem conductance measured when 50 mM KCl was added to the fluid perfusing samples first equilibrated with deionized water (upper panel) or with a 1 mM $CaCl_2$ solution. Error bars represent 1 SE (*n*=5).

Pinus samples perfused with deionized ultra-pure water or with a 50 mM KCl solution displayed the same patterns but P_1, P_2, and P_3 values were significantly lower in the presence of K^+ ions in the solution (Fig. 8). This suggests that pit membrane flexibility was increased by the presence of KCl in the fluid. The hydraulic conductance of *Fagus* samples, either perfused with water or KCl, remained constant for fluid pressures in the range of 0–1.4 MPa (Fig. 7).

Discussion

The main objective of this study was to test, on a diverse panel of tree species, if a modulation of xylem hydraulic conductance by the sap ionic composition had an impact on conduit vulnerability to cavitation, both parameters being thought to be related to pit membrane properties. In the positive, this would suggest that trees could control, in the short term, the drought resistance of their vascular system, just as they are thought to adapt their hydraulic efficiency (Zwieneckii *et al.*, 2001).

Ionic effects on xylem conductance

Sap ionic composition had a strong effect on xylem hydraulic conductance for most of the species evaluated in

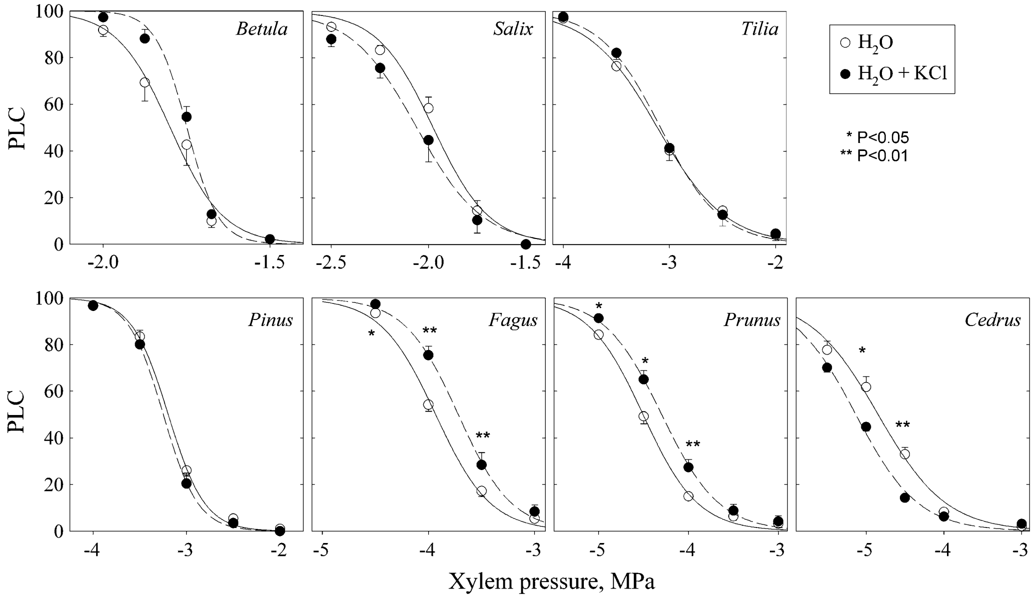

Fig. 4. Xylem vulnerability curves for samples of seven species perfused with deionized water (open circles) or with a solution of 50 mM KCl in deionized water (closed symbols). Error bars represent 1 SE (*n*=5).

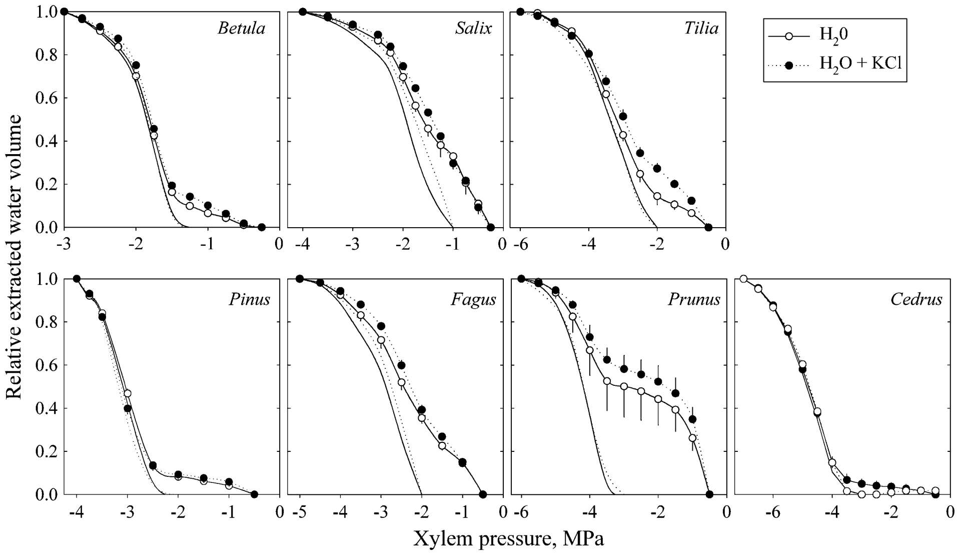

Fig. 5. Xylem water retention curves for samples of seven species perfused with deionized water (open circles, solid lines) or with a solution of 50 mM KCl in deionized water (closed symbols, dotted lines). The lines without symbols are corrected retention curves taking into account only the water released beyond the point of xylem cavitation. Error bars represent 1 SE (*n*=3).

this study. However, the results were much contrasted between species, and only *Tilia* responded typically like the species documented by previous authors (Zwieniecki *et al.*, 2001), i.e. a decrease in xylem conductance during perfusion with pure water and a sharp increase with the addition of K^+ cations in the solution. The current interpretation of this phenomenon is attributed to both the porous structure and the high pectic content of inter-vessels pit membranes. The swelling behaviour of pectins is thought to affect the size of microchannels in pit membranes through which water moves, and hence their resistance to water flow. A perfusion with deionized water could provoke an efflux of calcium and other counter-ions which would increase pectins swelling and thus would reduce xylem hydraulic conductance. Conversely, a perfusion with K^+ cations could alleviate the Donnan effect which would lead to the shrinking of pectins and an increase in conductance. The presence of Ca^{2+} cations in the reference fluid could inhibit both pure water and K^+ effects because it could buffer the variations in the Donnan effect and therefore the swelling of pectins.

This typical behaviour did not apply to all the species documented in this study, which confirms the recent observations of Nardini *et al.* (2007*a*). There are actually many reasons that could explain drastically different patterns. First, the chemical composition of pectins at the pit membrane level is highly variable, both at the intra- and interspecific levels, pectins being 'the most complex macrostructure in nature' (Vincken *et al.*, 2003). Pectin structure clearly influences their physico-chemical properties and particularly their ability to shrink or swell in the presence of different ions in the solution (Willats *et al.*, 2001*b*; Zsivánovits *et al.*, 2005). Second, the ionic composition and concentration of the solution bathing the pectins strongly determine their swelling/shrinking properties either by counter-ion condensation and or by network cross-linking

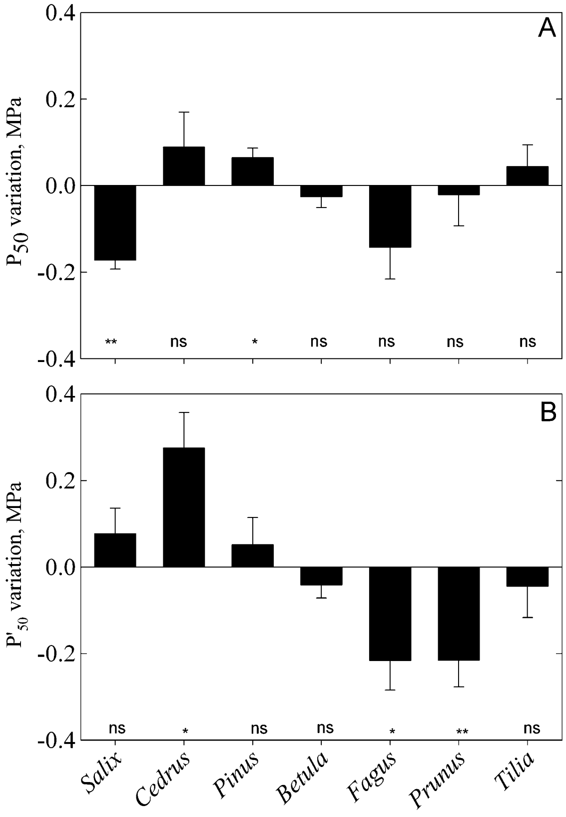

Fig. 6. Changes in vulnerability to cavitation values between segments perfused with deionized water or a solution with 50 mM KCl. Vulnerability to cavitation was measured as the xylem pressure provoking 50% loss of conductance (P_{50}, upper panel) or as the xylem pressure releasing 50% of sample water content (P'_{50}, lower panel). A positive variation signifies that samples perfused with KCl were less vulnerable to cavitation (more negative P_{50}) than control segments. Error bars represent 1 SE (*n*=3–5).

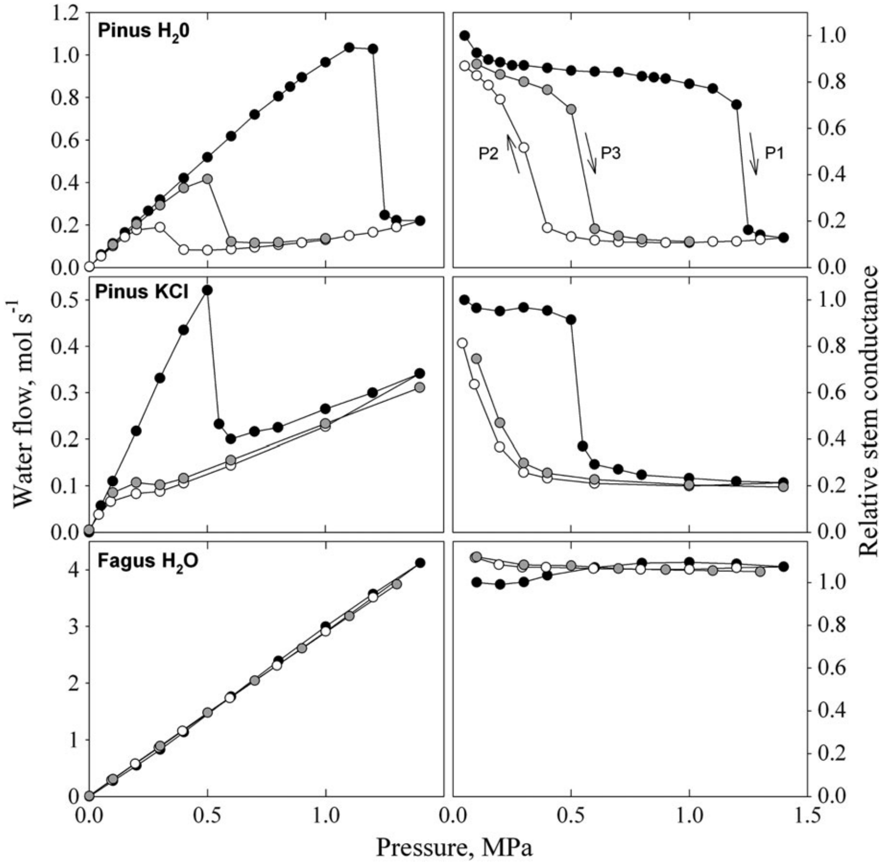

Fig. 7. Changes in water flow (left panels) and conductance (right panels) of xylem segments exposed to successive pressure cycles. Pressure was first increased from 0 MPa to 1.4 MPa (closed symbols), then returned to zero (open symbols) and finally increased again to 1.4 MPa (grey symbols). Representative curves are shown for *Pinus* segments perfused with deionized water (upper panel) or deionized water with 50 mM KCl (middle panel) and a *Fagus* segment perfused with deionized water (lower panel).

(Tibbits *et al.*, 1998; Zsivánovits *et al.*, 2005). In a more mechanistic point of view, the swelling/shrinking behaviour of pectins depends on three parameters: (i) the density and distribution of charges along the pectin network, i.e. the degree and repartition of methyl esterified carboxyl residues; (ii) the Ca^{2+} concentration and its distribution in pectins that cross-link pectin chains in the eggbox model; and (iii) the concentration and nature of counter-ions in the external solution. Only one KCl concentration (50 mM) was used in our study and it is possible that species may have responded to this single KCl concentration in different ways. However, for laurel (Gascó *et al.*, 2006) and chrysanthemeum (van Ieperen and van Gelder, 2006) the ion-mediated response was maximum at this concentration. Furthermore, for a particular counter-ion at a fixed concentration, it has been shown that pectin swelling increases depending on their degree of methylesterification (Zsivánovits *et al.*, 2005). The ion-mediated modulation of xylem conductance is also dependent on the size of the vessels (Gascó *et al.*, 2007), since more pit membranes are crossed by the ionic solution in stems with shorter conduits.

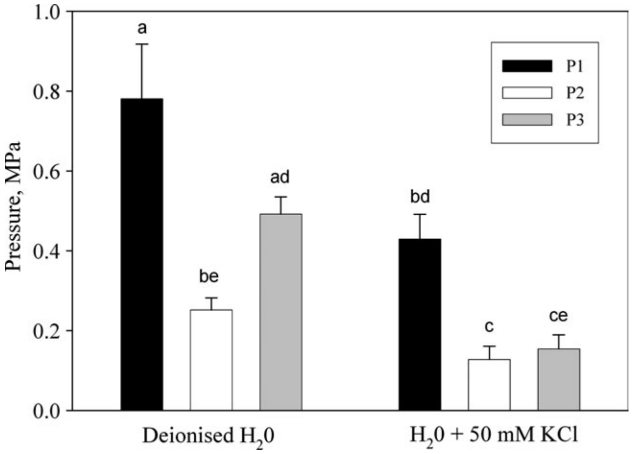

Fig. 8. Effect of KCl on *Pinus* pit membrane flexibility. Membrane flexibility was estimated by the perfusion pressure provoking 50% loss of *Pinus* sample conductance during successive pressure cycles. P1 corresponds to the first pressurization, P2 to the pressure relaxation phase, and P3 to the second pressurization. Error bars represent one SE (n=6). Treatments having a letter in common are not significantly different at P=0.05.

Another point of debate relates to the presumed porous structure of pit membranes which is central to the current understanding of the processes. The structure of pectins is strongly influenced by their degree of hydration. Until now, pit membranes have been observed on totally anhydrous material using conventional scanning electronic microscopy techniques. This may explain why they were visualized as porous structures (Choat *et al.*, 2003). By contrast, hydrated pectins may form a more or less continuous gel between the cellulosic microfibrils in the pit membrane, which may imply a much less porous structure. This hypothesis is supported by recent observations of hydrated pit membranes in the wood of *Sapium sebiferum* with an atomic force microscope demonstrating the presence of a thin mucilaginous layer covering the entire surface of the membrane (Pesacreta *et al.*, 2005). The transition between solid and gel states in pectins is influenced by the ionic strength and the degree of methylesterification (Garnier *et al.*, 1993; Goldberg *et al.*, 1996). Hence, the prevalence of the gelled versus the porous structure in pit membranes can also depend on the ionic strength of the fluid. Moreover, the degree of hydration of a pectin gel is also known to influence strongly its permeability to water (Tibbits *et al.*, 1998), more hydrated gels being more permeable. It can then be seen that the degree of pectin hydration will have opposite effects on its permeability if pit membranes have a porous or a non-porous structure. Structural investigations on hydrated pits with modern electronic microscopy techniques (ESEM FEG, AFM) are clearly awaited to clarify the exact structure of pits. For all the reasons listed above, the ion-mediated regulation of xylem hydraulic conductance is expected to be a very variable and partially unpredictable phenomenon (Gasco *et al.*, 2007; Nardini *et al.*, 2007*b*). It is not surprising to find much contrasted xylem hydraulic responses to KCl and $CaCl_2$ solutions across species as already suggested by Nardini *et al.* (2007*a*). Some very speculative explanations for the different behaviours observed on the species tested in this study are given below.

For *Betula* or *Prunus* xylem conductance was not modified when segments were perfused with pure water or with KCl. This could be explained by low pectin contents in their pit membranes or by the peculiar chemical properties of these pectins. The increase in hydraulic conductance in *Fagus* following a perfusion with pure water cannot be explained with the porous pit structure model proposed by Zwieniecki *et al.* (2001), but would be more consistent with a gelled non-porous structure where an increase in gel water content would facilitate the water flow across the pit membrane. In *Salix*, *Cedrus*, and *Pinus*, a surprising decrease in hydraulic conductance was found induced by a perfusion with 50 mM KCl. A decrease in conductance has already been observed when perfusing the xylem of halophyte species with high NaCl concentrations, whereas lower concentrations induced an increase in the conductance (López-Portillo *et al.*, 2005). The saline concentration (about 560 mM) used in this study is much higher than the KCl concentration (50 mM) tested here, but glycophyte species such as *Salix*, *Cedrus*, and *Pinus*, may respond to a lower salt concentration than halophytes. Moreover, K^+ cations are more efficient than Na^+ cations in neutralizing negative charges from GalA carboxyl residues (Shomer *et al.*, 2003), suggesting that a decrease in conductance could be obtained with a K^+ concentration lower than Na^+ concentration. If pit membranes have a gelled non-porous structure, then increasing the ionic strength of the solution surrounding the gel could dehydrate the pectins and therefore reduce the water permeability of the pit membrane. Differences in ionic effect between species may also reflect different binding strengths or different access of counter-ions to a greater number of negative charges.

More information on the structure and composition of pit membranes is clearly awaited to understand their function better. Nevertheless, it was possible to modulate pit conductance substantially by means of different sap fluid compositions and different species. This enabled us to document the main objective of our study, i.e. the effects on xylem vulnerability to cavitation.

Ionic effects on xylem cavitation and embolism

Sap ionic composition may impact xylem vulnerability for two reasons. First, the presence of ions in the solution may have a direct effect on the process of cavitation, i.e. the penetration of air bubbles through pit membranes. Second, ions may also have a more indirect effect on the level of embolism, i.e. the degree of loss of xylem conductance caused cavitation (Gascó *et al.*, 2006). Therefore, two techniques were used to investigate the effects of ions in the sap on both the vulnerability to cavitation and the degree of embolism.

The available direct techniques for assessing xylem cavitation [e.g. acoustic emissions (Tyree *et al.*, 1984) or cryo-SEM (Tyree and Cochard, 2003)] are either laborious or imprecise. In this study, a new indirect method has been used to detect cavitation based on the amount of water released during centrifugation. This provided a rather quantitative and precise indication of xylem vulnerability to cavitation. Of course, the cavitation of a large conduit releases more water, so it was important to compare samples as similar as possible. Using this technique, it is shown that the ion-mediated effect on the vulnerability to cavitation was undetectable for most of the species investigated and significant but small for only two species (*Salix* and *Pinus*). This indicates that the process of air-seeding through the pit membrane is generally not affected by sap ionic composition.

To analyse the effects of ions in the sap on xylem embolism, the changes in hydraulic conductance caused by the cavitation of the xylem conduits were detected. Despite the large ionic effects on xylem conductance, the results showed that xylem vulnerability to embolism was not modified by sap composition for most species. Only three species (*Fagus*, *Prunus*, and *Cedrus*) showed significant, but minimal, variations. These small variations in embolism were only marginally caused by a change in vulnerability to cavitation as has been shown prevously, and more probably due to the change in xylem conductance itself (Gascó *et al.*, 2006). Indeed, when cavitation forms in a xylem conduit, the axial sap pathway is disrupted, but water can still move upwards because of radial connections between different conduits. Ions in the sap can strongly influence the conductance of these radial pathways. Hence, the overall loss of xylem conductance caused by a given percentage of cavitated conduits can be much reduced in the presence of an ion-mediated increase of hydraulic conductance. This would result in a more negative P_{50} value. This interpretation is consistent with the overall opposite variations in P_{50} and conductance values found in this study, although the relationship between the two parameters was poor across species. For instance, the vulnerability of *Fagus* was significantly affected by the presence of K^+ ions while its conductance was only slightly affected. By contrast, the vulnerability of *Tilia* was not modified by K^+ ions whereas its conductance was greatly enhanced.

Pit structure and function revisited

From our experiments on the effects of sap ionic composition on xylem conductance and xylem vulnerability to cavitation it can be concluded that the two parameters are largely uncoupled. In other words, the processes governing the water flow and gas penetration through pit membranes are also uncoupled. Our initial hypothesis was that the changes in pit conductance were caused by variations in pit membrane porosity and, hence, an effect on pit resistance to air-seeding was expected, as this process is also determined by pit membrane porosity. The explanation of our unexpected finding probably resides in the structure of pit membranes and on its consequence on the process of cavitation. When water flows through a pit, the pressure difference across the membrane is very small (in the order of a few kPa), and the membrane rests unstretched in the middle of the pit chamber. Pit porosity is then minimal and pectins possibly form a continuous gel between the cellulose strands. Under these conditions, a small swelling or shrinking of the pectin polymers will have a large impact on pit hydraulic conductivity. By contrast, during the process of air-seeding, the pit membrane is exposed to a very large pressure difference (in the order of several MPa) and the membrane must undergo substantial stretching and deformations. If the pores are enlarged during stretching, the ion-mediated variation in swelling of pectin would probably have a marginal effect on pore size. This hypothesis is consistent with recent studies on pit membranes demonstrating that the porosity of relaxed pit membranes is much less than the porosity derived from the Young–Laplace equation (Shane *et al.*, 2000; Choat *et al.*, 2004, 2008). The 'rare pore' hypothesis for cavitation induction (Christman *et al.*, 2009) would also be consistent with the results of our study. Under this hypothesis, the large pores responsible for air-seeding are too rare to influence xylem hydraulic conductance which is more controlled by the average pore size (Choat and Pittermann, 2009). If the pectin coating of cellulose microfibrils were relatively thin, then swelling/shrinking of the gel phase would be expected to have little effect on these large pore sizes.

For conifers, the situation is different because pit structure is also different. Here, the elasticity of microfibrils in the pits is thought to be the determining factor for the process of cavitation (Hacke *et al.*, 2004), with species more resistant to cavitation having more rigid microfibrils. In *Pinus*, pit membrane flexibility was significantly increased in the presence of KCl in deionized water. However, no significant differences were found in the P_{50} values for this species. This suggests that microfibril elasticity alone does not explain the difference in cavitation resistance across conifers.

Conclusion

The presence of ions in the sap considerably influenced the hydraulic conductance of the xylem conduits for the different species documented in this study. However, the effects were much contrasted across species suggesting that the underlying mechanisms are probably more complex than initially proposed. Investigations on hydrated pit membranes are needed to understand the difference between species. Despite very large variations in xylem conductance, we failed to detect any substantial effect on vulnerability to cavitation. This demonstrates that the two phenomenons are probably mechanistically decoupled. The functional significance of ion-mediated changes in xylem conductance is still debated (van Ieperen and van Gelder, 2006; Nardini *et al.*, 2007*b*) but, from our study, it seems clear that trees have a very limited ability to modulate their xylem vulnerability by adjusting their sap ionic composition. The benefit trees may gain by increasing their xylem conductance is hence not jeopardized by an increase in vulnerability to cavitation.

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