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Water content, hydraulic conductivity, and ice formation in winter stems of *Pinus contorta*: a TDR case study

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Abstract Stem water content, ice fraction, and losses in xylem conductivity were monitored from November 1996 to October 1997 in an even-aged stand of *Pinus contorta* (lodgepole pine) near Potlatch, Idaho, USA. A time domain reflectometry (TDR) probe was used to continuously monitor stem water contents and ice fractions. Stem sapwood water contents measured with TDR were not different from water contents measured gravimetrically. The liquid water content of stems ranged from 0.70 $m^3\ m^{-3}$ to 0.20 $m^3\ m^{-3}$ associated with freezing and thawing of the wood tissue. Ice fraction of the stem varied from 0–75% during the winter suggesting liquid water was always present even at ambient temperatures below –20°C. Shoot xylem tensions decreased through the winter to a minimum of ca. –1.4 MPa in February then increased to –0.4 MPa in May. Shoot xylem tensions decreased during the growing season reaching –1.7 MPa by September. Annually, low shoot water potentials were not correlated to decreases in stem hydraulic conductivity. Xylem conductivity decreased due to cavitation through the winter and was 70% of summer values by March. Decreases in xylem conductivity were correlated to low shoot water potentials and cumulative freezing and thawing events within the xylem. Xylem conductivity increased to pre-winter values by May and no reductions in xylem conductivity were observed during the growing season.

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Introduction

Terrestrial plants depend on the transport of water from the soil through the plant for growth and survival. Stresses such as low xylem water potentials, freezing and thawing of the xylem water, and some pathogens may disrupt the vital processes of xylem transport by inducing air obstructions known as embolisms (Newbanks et al. 1983; Crombie et al. 1985; Salleo and Lo Gullo 1989). Embolisms are initiated by a process known as cavitation where air bubbles are aspirated into xylem conduits due to low xylem water potential (Zimmermann 1983; Grace 1993; Pockman et al. 1995; Sperry et al. 1996). Although low xylem water potentials are usually associated with summer drought conditions, relatively little cavitation has been reported during the growing season in temperate trees (Sperry et al. 1988; Sperry and Sullivan 1992; Sperry et al. 1994). In contrast, cavitation in temperate trees during the winter can be extensive (Sperry and Sullivan 1992; Wang et al. 1992; Sperry et al. 1994).

Reduction of hydraulic conductivity during the winter may result from a second cavitation mechanism proposed by Hammel (1967). That is, freezing of the xylem water forces dissolved gases out of solution to form bubbles in the ice. On thawing, these bubbles can either dissolve back into the xylem water or they can grow to obstruct the entire xylem conduit (Zimmermann 1983). The formation of an embolism upon thawing depends on the size of the bubble and the water potential of the xylem; with larger bubbles and lower water potentials most likely forming an embolism (Yang and Tyree 1992). Therefore, susceptibility to freeze-induced cavitation is related to conduit size because larger bubbles may form in larger conduits (Sperry and Sullivan 1992).

Coniferous trees exhibit some of the smallest conduit diameters of any temperate tree (Ewers 1985). The increased resistance to freeze-induced cavitation associated with small diameter conduits has been proposed to allow conifers to dominate freezing environments (Sakai and Larcher 1987). Although conifers are thought to be highly resistant to freeze-induced cavitation, losses of hydraulic conductivity in conifers during the winter have been observed to be 20–50% (Sperry and Sullivan 1992; Sperry et al. 1994; Sparks and Black 2000). In addition, cavitation has not been detected in controlled laboratory measurements following several freeze-thaw cycles (Sperry and Sullivan 1992) or in experiments where freezing and low water potentials were applied simultaneously using a centrifuge and freezing system (Davis et al. 1999). Further, Sperry et al. (1994) found no correlation in the field between cumulative freeze-thaw events and cavitation for a number of conifer species. Based on these studies, conifers apparently experience some level of cavitation during the winter, but the causative factor or factors are elusive.

Earlier studies of winter water relations in conifers (e. g. Sperry et al. 1994; Sparks and Black 2000) were limited by our inability to continuously monitor volumetric water content and ice fraction of the wood during winter months. Continuous measurements of wintertime water and ice contents of conifer wood would address the combined effects of wintertime desiccation and ice formation on hydraulic conductivity. In the present study we use time domain reflectometry (TDR) to measure the annual patterns of water content and ice formation in conifer xylem. We hypothesize wintertime stem water contents, wood ice contents, xylem water potentials, and freezing and thawing events within the xylem may contribute to changes in wintertime xylem hydraulic conductivity in *Pinus contorta* Dougl. ex Loudon (lodgepole pine). The objectives of this study were: (1) make the first in situ measurement of ice formation in the wood of coniferous trees, (2) measure the annual pattern of stem volumetric water content, ice content, shoot water potential, and xylem hydraulic conductivity, (3) determine the vulnerability of the xylem of *P. contorta* to cavitation in the absence of freezing using air injection, and (4) to investigate the relationships between changes in wintertime hydraulic conductivity and environmental conditions.

Materials and methods

Research site and plant culture

Measurements were made on approximately 200 trees of *P. contorta* growing in an even aged stand on the University of Idaho Experimental Forest near Potlatch, Idaho USA (46° 91′N and 116° 32′ W). All trees used for study were 18–22 cm in diameter (DBH) and were approximately 12 years old.

Laboratory methods: vulnerability to tension-induced xylem cavitation

We investigated the resistance of the xylem of *P. contorta* to cavitation in the absence of freezing stress by generating vulnerability curves following the procedures described by Cochard et al. (1992) and Sperry and Saliendra (1994). Vulnerability curves were generated for two branch segments from each of five individual trees of *P. contorta* randomly selected from the research site on 12 July 1996. Stem segments greater than 1 m in length and 11–13 mm in diameter were harvested, returned to the laboratory, and re-cut to between 0.2 and 0.25 m under water to avoid introducing additional embolisms. As recommended by Sperry and Saliendra (1994), small-notched cuts were made in each segment to increase air access to the vascular system. The segments were then enclosed in a double-ended pressure chamber so the ends of the stem segment protruded from each end of the chamber. The proximal end of the segment was then attached to a low-pressure head of water with clear Tygon tubing. Filtered (0.2 µm) water, adjusted to pH 2 to avoid microbial growth, was passed through the stem at low pressure (approximately 0.01 MPa). Hydraulic conductance (k) was defined as the mass flow rate of water through the stem divided by the water-pressure head.

We generated a vulnerability curve by first pressurizing the air chamber to 0.05 MPa to avoid water extrusion from side branches or the notched cuts, and equilibrated the system for 10 min. Once equilibrated, flow of water through the stem was increased and the initial conductance (k_i) through the stem was measured by collecting effluent from the distal end of the stem until the measurements were repeatable. After the initial measurement, the pressure head of water was lowered and the chamber air pressure raised by preset increments (approximately 0.25 MPa) and allowed to equilibrate for 10 min. After equilibration, the air chamber pressure was reduced to 0.05 MPa, the water flow increased, and the conductance measured (k_m). This process was repeated until conductance of the segment was negligible.

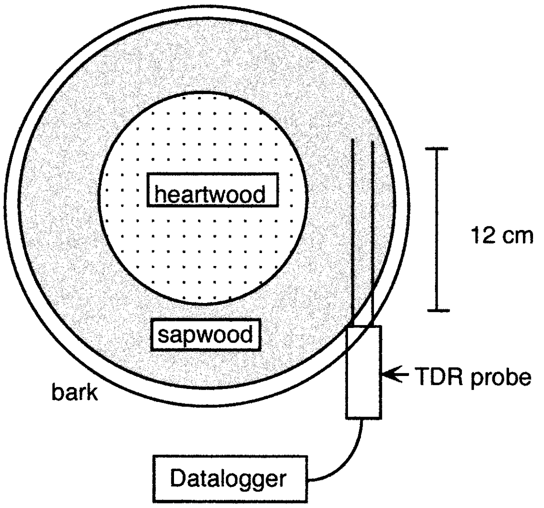
TDR measurements

TDR measures the apparent dielectric constant (i.e., the resistance of the material to the development of an electric field) around a transmission line inserted into a media. Davis (1975) first described a method using TDR to measure the volumetric water content of a porous medium. Topp et al. (1980) and Topp and Davis (1985) demonstrated there is a linear relationship between the apparent dielectric constant and the water content of a porous medium using TDR measurements of several types of porous material. Spaans and Baker (1995) have extended this work to show that changes in liquid water content within a porous medium during freezing is similarly linearly related to the TDR measurement.

A unique calibration curve for lodgepole pine and a standardized probe orientation to maximize contact with the sapwood were developed to avoid errors associated with non-uniform distribution of water in the stem. Stems differ from other porous media in that trees tend to exhibit higher water contents in the outer sapwood layers compared to the inner heartwood layers (Constantz and Murphy 1990).

A calibration curve was developed on a wood segment from a representative tree of *P. contorta* collected from the study area. The wood segment was from the outermost part of the bole, was primarily sapwood with a total volume of approximately 1,200 cm^3. In this and all subsequent measurements, a TDR probe (water content reflectometer, Model CS615, Campbell Scientific, Logan, Utah) with two stainless steel rods (12 cm long, 0.32 cm diameter, and 3 cm separation) was driven into parallel pilot holes drilled into the sapwood. To standardize the orientation of the probe in the stem we chose a characteristic stem diameter of 20 cm and built a probe-insertion guide using a Plexiglas frame with guide holes. Using this guide, pilot holes were drilled tangential to the annual increment of wood and well within the sapwood (Fig. 1, panel A). The TDR probe measures the integrated water content (m^3 water m^{-3} wood) in a cylindrical wood volume with a diameter of 6 cm (twice the rod separation) and 12 cm in length. The rod length of 12 cm was used because previous investigations had shown that longer probe lengths were difficult to insert into the stem (Constantz and Murphy 1990). The TDR probe was connected to a Campbell Scientific CR10 datalogger and data were recorded at 2-min intervals.

A. cross section of tree stem



B. Calibration curve

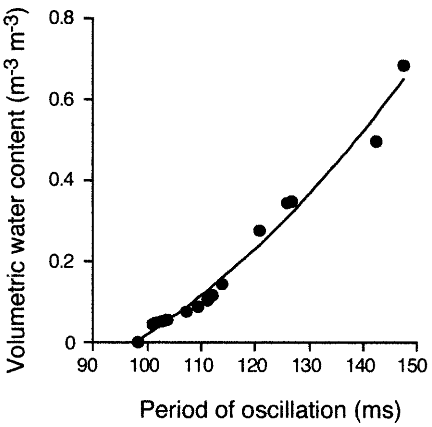


Fig. 1 **A** A schematic for laboratory and field measurements of stem water and ice content using the time domain reflectometry (TDR) probe. Orientation of the probe into the sapwood was standardized among trees using a probe insertion guide made of Plexiglas. **B** Relationship between gravimetric volumetric stem water content and the TDR measurement for wood of *Pinus contorta*. The array of data points were fit with a second-order polynomial (VWC $=1.01\times10^{-6}\theta^2 - 1.16\times10^{-4}\theta + 0.175$, r^2=0.912) where VWC is the volumetric water content and θ is the probe oscillation time in microseconds. This relationship was used as a calibration for all subsequent TDR measurements

For calibration, the wood segment was saturated and then dried on a benchtop while measurements of water content were made using a balance and the TDR probe. Additionally, eleven core samples were taken at random intervals during drying and analyzed gravimetrically. Following measurement, the wood segment was dried at 70°C to a constant weight. The volume of the wood segment was determined using a water displacement technique and volumetric water content was calculated.

The initial volumetric water content of the wood segment was 0.69 $m^3\ m^{-3}$ and dried to 0.05 $m^3\ m^{-3}$ over 3 weeks. Oscillation times of the TDR probe were correlated (Fig. 1, panel B) to volumetric water content by a second order polynomial of the form:

$$VWC = 0.175 + 1.00 \times 10^{-6}\,\theta^2\ 1.16 \times 10^{-4}\,\theta$$

where VWC is the volumetric water content ($m^3\ m^{-3}$) and θ is the period of signal oscillation (μs). Because changes in liquid water content during freezing have been shown to behave similarly to changes in liquid water during drying (Spaans and Baker 1995), we also used this calibration to estimate unfrozen water in the stem during the winter.

Water contents of the wood segment estimated by TDR were in close agreement with gravimetric measurements of increment cores (slope =0.983, r^2=0.923, $P<0.001$, n=11).

Field measurements

The TDR system was installed in a 20-cm-diameter lodgepole pine at the University of Idaho Experimental Forest on 20 November 1996 using the procedure described above for laboratory measurements. Temperature measurements of the wood were made concurrent with each TDR measurement using fine-wire thermocouples on the surface of the 20 cm stem, at the measurement depth for the TDR probe (approximately 3 cm), and embedded into the sapwood of two 1-cm-diameter stems. Ambient temperatures were measured 1 m above the soil surface and 1 cm below the soil surface using fine-wire thermocouples. All TDR and temperature measurements were monitored at 2-min intervals at the site using a Campbell Scientific CR10 datalogger. Precipitation was measured using the permanent weather station in Potlatch, Idaho approximately 6 km from the research site.

The ice fraction of the wood was estimated using the following equation:

$$\text{Ice fraction} = 1 - \left(\frac{VWC_a}{VWC_t}\right) \qquad (1)$$

where VWC_a is the measured volumetric liquid water content and VWC_t is the total water content (liquid and ice phases within the stem). VWC_t was estimated using the TDR measurement made at the nearest ice-free time period. An ice-free period was defined as 8 consecutive hours over 1°C measured by the thermocouple embedded with the TDR probe.

Volumetric water content of the xylem of 14 trees was measured gravimetrically every 2 weeks during the experiment. Trees used for gravimetric analyses were within 40 m of the tree used for the TDR measurement and were of similar height and DBH. Volumetric water content was measured for branch segments (1 cm in diameter) and for samples cored from the heartwood and sapwood from the main axis stem. Heartwood was sampled from the innermost 1 cm of the core and sapwood the outermost 1 cm of the core adjacent to the cambium. Branch and core samples were weighed before and after drying at 70°C for 48 h. and volumes were estimated from physical measurements.

Hydraulic conductivity and loss of hydraulic conductivity were measured following the procedures of Sperry et al. (1988) on stems collected coincident with gravimetric measurements of volumetric water content. Fourteen stems greater than 1 m in length and 9–11 mm in diameter were collected in the field, sealed in plastic bags and returned to the laboratory. Stems were re-cut to 10-cm segments under water and mounted on a positive flow hydraulic conductivity apparatus. Hydraulic conductivity of each segment was measured before (K_i) and after removal of embolism (K_{max}) by high pressure (150 kPa) flushing of water through the xylem. The percentage loss of hydraulic conductivity was calculated as:

$$\%\ \text{loss in xylem conductivity} = 100(K_{max}-K_i)\ /\ K_{max}, \qquad (2)$$

where K_i is the initial conductivity for the segment and K_{max} is the maximum conductivity for the same segment after flushing.

Shoot water potentials were measured using a pressure chamber (P.M.S., Corvallis, Ore.). Summer measurements of shoot water potential were collected at predawn and solar noon in the field. Stem segments for winter measurements of shoot water potential were collected mid-day in the field, sealed in plastic bags and returned to the laboratory, allowed to thaw and measured at room temperature. The wintertime measurements of shoot water potential, therefore, represent a relative index comparing water status across all sampled stems and may or may not be a direct measurement of ‘in situ’ shoot water potential.

Statistical analysis

Linear regression analyses were performed using a general linear model (GLM) procedure (SAS) (Littel et al. 1991). Vulnerability curves were analyzed using tests analogous to determining the air-entry point (ψ_e) in soils under increasing tensions (Brooks and Corey 1966; Campbell 1974; Sparks and Black 1999). In soils, the air-entry point is defined as the tension (or suction) where the macro-pore spaces within the soil become air filled and hydraulic conductivity decreases rapidly. Similarly in plants, vulnerability curves exhibit a point, defined here as the air-entry point, beyond which hydraulic conductivity decreases rapidly (Edwards and Jarvis 1982; Zimmermann 1983). A log/log plot of the relative conductivity (k/k_{max}) against the absolute value of the stem water potential results in two distinct intersecting lines before and after an abrupt change in slope. A power function fit of the line after the intersection point gives a linear relationship of the form:

$$k/k_{max} = a|\psi|^b \qquad (3)$$

where k/k_{max} is the relative conductivity, ψ is the tension applied to the system, and a and b are fitting parameters. By rearranging and solving this equation for maximum conductance (i.e., k/k_{max} =1) we can calculate the air-entry point (ψ_e):

$$\psi_e = -(1/a)^{1/b} \qquad (4)$$

The air-entry point does not define the point where hydraulic conductivity begins to decrease. It does, however, identify where the rate of loss of conductivty per unit pressure increases. We hypothesize the air-entry point represents the xylem tension where pit membranes are completely overcome and 'runaway' xylem cavitation begins. Air-entry points measured in the angiosperm species *Populus trichocarpa* were shown to approximate the leaf water potential where stomatal conductances decreased during drought treatments presumably to avoid damage to the conductive tissue (Sparks and Black 1999). Air-entry points are used in the present study as an index to compare vulnerabilty curves to field observations.

Results

The TDR technique provided field estimates of sapwood water content that were in close agreement with gravimetric measurements (Fig. 2, open circles). However, TDR underestimated volumetric water content by a maximum of 0.03 m^{-3} m^{-3} during some sampling periods compared to gravimetric measurements (<5% error). Because the TDR probe was installed only in sapwood, the gravimetric estimates of water content of the heartwood (Fig. 2, open triangles) and canopy stem segments (Fig. 2, open circles) were not correlated to the TDR measurements. Additionally, changes in sapwood water contents measured by TDR were positively related to shoot water potential (Fig. 3, r^2=0.492, P<0.05, n=30) implying changes in bole water contents were related to shoot water status.

Sapwood ice fraction of the stem varied from 0 to 75% between November 1996 and April 1997 (Fig. 4b). Regardless of the ambient temperature, the sapwood at the point of TDR measurement always contained more than 25% liquid water. Stem temperatures and ice contents were not correlated. Often, the temperature of the stem remained relatively constant near 0°C (Fig. 4a) while the ice fraction fluctuated as much as 75% (Fig. 4b).

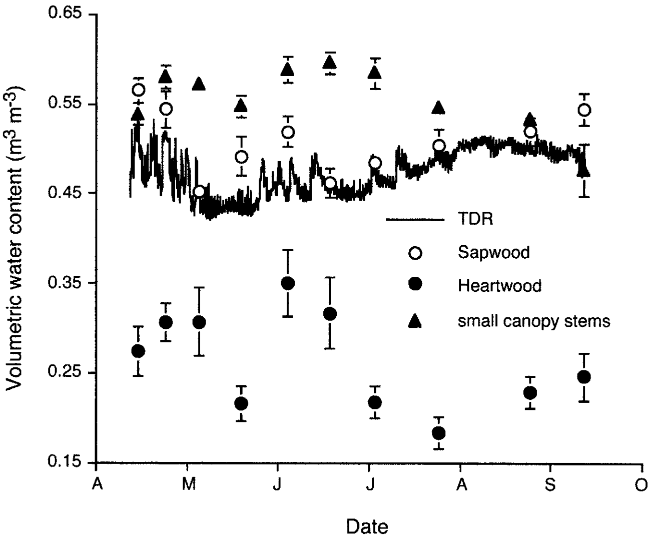


Fig. 2 Gravimetric measures of volumetric water content and the TDR measurement of volumetric water content for sapwood of *P. contorta* over a 6-month period in 1997. The *solid line* represents the continuous measurement of stem water content by TDR. *Open circles* represent the volumetric water content of sapwood samples. *Closed circles* represent the volumetric water content of heartwood samples. *Closed triangles* represent the volumetric water content of 1 cm diameter stem segments taken from the canopy. *Error bars* are ± 1 SE

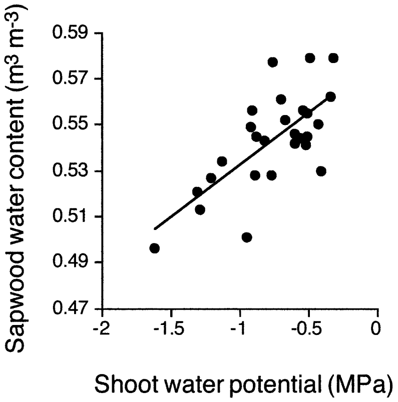


Fig. 3 Relationship between sapwood water content measured from cores and shoot water potentials measured on the same tree. The parameters are positively correlated (r^2=0.492, P<0.05, n=30)

The liquid water content of the main stem varied from over 0.70 m^3 m^{-3} to less than 0.20 m^3 m^{-3} during the winter (Fig. 5a). The range of water contents in the stem observed during the winter coincided with cycles of temperatures above and below freezing within the wood. Measurements of wood temperature suggested the wood completely thawed (here defined as 8 consecutive hours over 1°C at the point of TDR measurement) at least seven times during the winter (Fig. 5a, numbered arrows). Among these periods of thaw, the volumetric water content of the stem varied between 0.72 m^3 m^{-3} and 0.49 m^3 m^{-3}.

Shoot water potentials decreased from –0.4 to –1.3 MPa during winter months (Fig. 5b). Over this

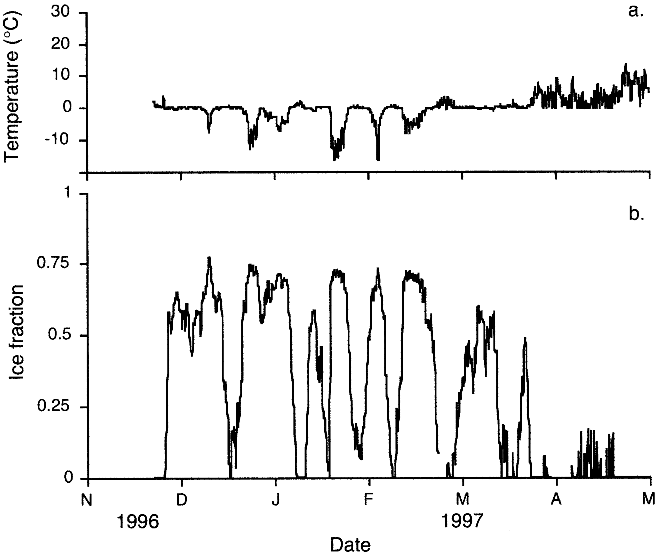


Fig. 4 Trend in **a** wood temperature, and **b** ice fraction of a 20-cm-diameter stem from November 1996 to May 1997. Ice content of the sapwood is shown as a fraction with 0 designating that all water in the stem was liquid and 1 designating all stem water was ice

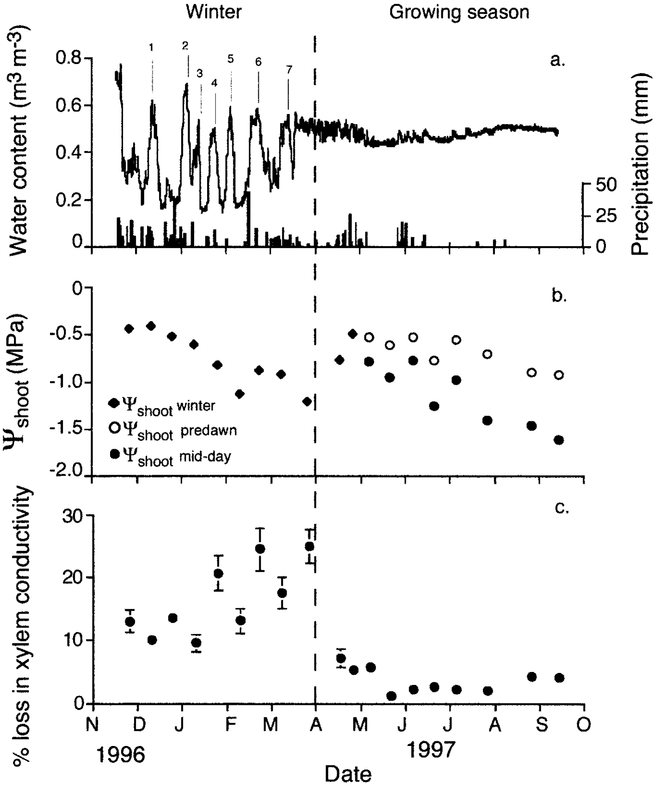


Fig. 5 Trends in sapwood water content (*solid line*) and precipitation (*solid bars*) (**a**), xylem water potential (**b**), and loss in xylem hydraulic conductivity (**c**) from November 1996 to October 1997 for an individual *P. contorta* growing in the University of Idaho Experimental Forest. The *dashed line* separates the panels into winter (November-April) and growing season (May-October) measurements. Seven mid-winter thaw events are shown by numbered arrows. Xylem water potential was measured once daily at 2-week intervals during winter months (*closed diamonds*) and at predawn (*open circles*) and solar noon (*closed circles*) at 2-week intervals during the summer months. Each data point for xylem water potential and hydraulic conductivity is a mean of fourteen trees. *Error bars* are ± 1 SE. *Error bars* are not shown if smaller than the symbol used

same period, stem hydraulic conductivity was reduced between 10 and 23% (Fig. 5c). During the summer, predawn shoot water potentials ranged from –0.5 MPa in May to –0.9 MPa in September (Fig. 5b) and mid-day shoot water potentials varied from –0.7 MPa in May to –1.7 MPa in September. Losses in xylem conductivity did not exceed 5% from May to September (Fig. 5c).

TDR measurements of stem water content during the growing season varied from 0.54 m^3 m^{-3} in early April to 0.43 m^3 m^{-3} in mid-May (Fig. 5a). The highest stem water contents (>0.50 m^3 m^{-3}) were observed during warm periods in the winter and in early April following snowmelt. After snowmelt, stem water contents decreased to 0.43 m^3 m^{-3} associated with a 30-day period of no precipitation in late May and early June (Fig. 5a). Stem water contents were relatively constant (0.44 m^3 m^{-3} to 0.48 m^3 m^{-3}) from June to October.

Decreases in stem hydraulic conductivity were correlated to shoot water potential and freezing and thawing events in the xylem (Fig. 6). Stem hydraulic conductivity decreased as shoot water potentials became more negative (Fig. 6a, r^2 =0.481, P<0.05) and repeated freezing and thawing of the xylem was positively correlated to decreased stem hydraulic conductivity (Fig. 6b, r^2 =0.577, P<0.05). Hydraulic conductivity did not decrease associated with low stem water contents or low shoot water potentials during the summer months.

A multiple regression analysis was used to investigate the combined effect of shoot water potential and freeze-thaw cycles on stem hydraulic conductivity during the winter. No interaction between shoot water potential and cumulative freezing events was detected (F=2.07, NS) in their effect on loss of hydraulic conductivity, and we combined both parameters into a single model. Cumulative freezing events explained significantly more variation than shoot water potential (r^2=0.295, F=26.44, P<0.0001) and shoot water potential did not add to the predictive power of the model considering both parameters. Because shoot water potential and freezing events are correlated through time, both may affect changes in xylem conductivity.

Summer precipitation increased water content of the stems by more than 0.05 m^3 m^{-3} for 2–3 days on several occasions (Figs. 5a, 7). For example, stem water content increased 0.058% over a 12-h period following a 25 mm precipitation event on 23 May 1997 (Fig. 7). Stem water contents fluctuated approximately 2% diurnally during periods of active transpiration (Fig. 7). Afternoon increases in stem water content of approximately 0.005%, probably a result of stomatal closure, were observed every day of active transpiration during the experiment.

The relationship between stem liquid water content and wood temperature differed between winter and sum-

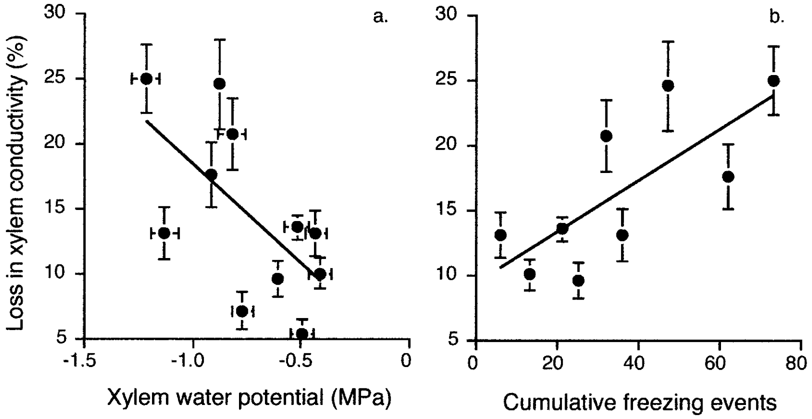


Fig. 6 Correlation between loss of xylem hydraulic conductivity and xylem water potential (r^2=–0.481, P<0.05, n=11) (**a**) and loss of xylem hydraulic conductivity and cumulative freeze-thaw cycles of the wood (r^2=0.577, P<0.05, n=9) (**b**). Only data for the winter months (November-April) were used in the correlations. Error bars are ± 1 SE. Data points are the mean of 14 values

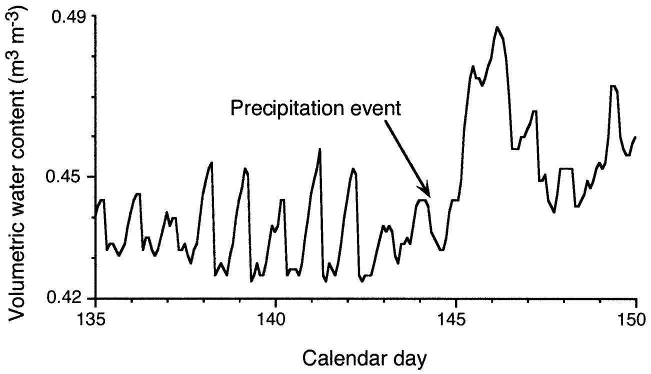


Fig. 7 Trend in the volumetric water content of sapwood over a 2-week period in late May as measured by TDR. Measurements were made at 2-min intervals before and after a 25 mm precipitation event on 24 May 1997 (day 144)

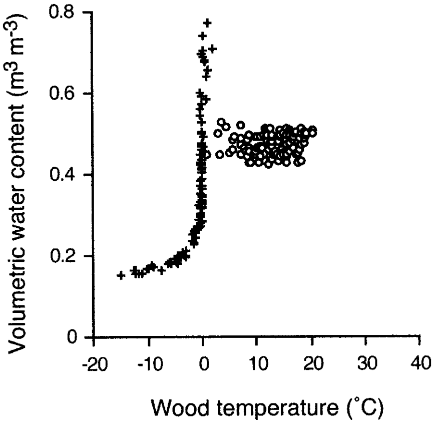


Fig. 8 Relationship between sapwood water content of a 20-cm-diameter stem as measured by TDR and wood temperature. Volumetric water content was measured at 2-h intervals during the winter months (*pluses*) and during the growing season (*open circles*). For clarity, only 5% of the total measurements are shown. Plots of the entire dataset did not alter the observed relationships

Table 1 Air-entry points (Ψ_e) in five individuals of *Pinus contorta* from the University of Idaho Experimental Forest. Error values of means are ±1 SE

Replicate	Ψ_e (MPa)
1	3.18±0.02
2	3.25±0.01
3	3.34±0.04
4	3.36±0.02
5	3.38±0.02
Mean ± SE	3.30±0.03

mer months (Fig. 8). During the winter, volumetric liquid water content of the stem varied from 0.20 to 0.80 m^3 m^{-3} at temperatures near 0°C (Fig. 8, pluses). At temperatures from 0 to –18°C, liquid water contents decreased from 0.20 to approximately 0.17 m^3 m^{-3} due to freezing of stem water. During the summer, water contents of the stem varied from approximately 0.45 m^3 m^{-3} to 0.55 m^3 m^{-3} with little relationship to wood temperature (Fig. 8, open circles). The highest volumetric water contents (>0.75 m^3 m^{-3}) of stems were observed in the winter at temperatures between 0 and 10°C.

The hydraulic conductivity for stems of *P. contorta* decreased under laboratory measurements that simulated decreased xylem water potentials in the absence of freezing. The Ψ_e for field collected stems of *P. contorta* was 3.30±0.03 MPa (Table 1, n=10). Water potentials lower than –3.3 MPa (i.e. the air-entry point measured in the laboratory) were never recorded in the field (Fig. 5b).

Discussion

Many researchers have investigated ice formation in conifer xylem using theoretical analyses (Zimmermann 1964; Hammel 1967; Sucoff 1969; Robson and Petty 1993) and microscopy (Robson and Petty 1987; Robson et al. 1988), but to our knowledge, ours is the first in situ measurement of ice formation in the wood of coniferous trees. The ice content of the xylem of *P. contorta* measured by TDR was found to be variable and often unrelated to wood temperature during winter months (Fig. 4). The use of wood temperature measurements to monitor freezing in the xylem may result in an overestimation of the frequency of freezing events and be dependent on water held in large pore spaces which freeze at temperatures between 0 and –1°C (Robson and Petty 1987). Further, measurement of ice formation in xylem elements of varying sizes or determining unfrozen water contents would require a temperature independent method such as TDR.

Perhaps the most interesting result of our measurements is the demonstration that more than 25% liquid water exists in the wood even at temperatures as low as –20°C. While the anatomical location of this liquid water is unknown, it is likely held within the cell wall material or within living cells. Conductivity of this liquid water has not been measured and is probably very small, but it has been speculated that such flow can occur in frozen tissue through cell wall capillaries (Hygen 1965; Havis 1970; Grace 1993). Our measurements suggest liquid water would be available for flow through such a mechanism.

Xylem of *P. contorta* in this study exhibited less than a 5% loss in xylem hydraulic conductivity over the growing season (Fig. 5c). Low losses of stem hydraulic conductivity during the growing season have been reported for other tree species (Sperry et al. 1988; Sperry et al. 1994) suggesting stomatal control generally prevents critical tensions from developing although xylem water potentials may approach values know to cause cavitation (Tyree and Sperry 1988; Sperry et al. 1993). In contrast to summer measurements, *P. contorta* lost up to 28% of its hydraulic conductivity during the winter (Fig. 5c). Similar losses of hydraulic conductivity during the winter have been observed in other evergreen conifers (Sperry et al. 1994) and have been attributed to low xylem water potentials, freeze-thaw cycles within the xylem, wintertime desiccation, or a combination of these factors.

The xylem of *P. contorta* apparently was not desiccated during the winter in this study. Stem water content varied among winter-thaw periods between 0.72 $m^3\ m^{-3}$ and 0.49 $m^3\ m^{-3}$ (Fig. 5a), but always remained greater than summer values of 0.44 to 0.48 $m^3\ m^{-3}$ (Fig. 5a). Although the stem water content of *P. contorta* did not decrease below 0.49 $m^3\ m^{-3}$ during the winter, there was variation (>20%) between thaw periods. Decreases in stem water content probably result from foliar evaporation and limited uptake of water from cold or frozen soils (Katz et al. 1989; Sperry 1993). The increases in stem water content we observed would require the availability and uptake of liquid water. Uptake of water through the root system seems unlikely because soil temperatures through the winter at 5 cm were generally below freezing where root conductivity is reported to be limited (Owston et al. 1972; Teskey and Hinckley 1981; Coleman et al. 1992). We offer direct water uptake by stems or foliage as an alternative explanation for increases in stem water content. Mid-winter thaws that exhibited increased stem water contents (e.g. Fig. 5a, peak 2) were often preceded by high levels of snowfall (Fig. 5, precipitation in late December). Conversely, mid-winter thaw periods exhibiting decreased stem water contents (e. g. Fig. 5a, peaks 3 and 4) were generally preceded by little or no precipitation. Finally, the highest stem water contents (i.e., water contents >0.75 $m^3\ m^{-3}$) were observed during the winter at temperatures between 0 and 3°C (Fig. 8). This implies that if snow were present in the canopy during the thaw, a portion of this water may be directly absorbed by the stems and foliage. Uptake of water from melting snow has been reported previously in *Picea abies* (Katz et al. 1989) and has been hypothesized to be important for maintaining winter water balance in conifers (Stone 1963). Our data support these earlier hypotheses and indicate direct uptake of melting water by stems or foliage may be an important mechanism to reduce winter desiccation.

Decreased shoot water potentials during the winter coincided with decreased hydraulic conductivity (Fig. 6a). However, our observations of the annual cycles of shoot water potentials indicate that negative water potentials in the xylem of shoots are not the sole cause of winter cavitation. Shoot water potentials ranged from –0.5 to –1.4 MPa during the winter months and from –0.4 to –1.7 MPa during the summer months (Fig. 5b). At no time during this annual cycle did shoot xylem tensions approach the air-entry point measured in the laboratory (–3.3 MPa, Table 1). In addition, the lowest shoot water potentials (–1.7 MPa) were observed during summer months when cavitation was negligible (<5%).

Relationships between cavitation during winter months and freeze-thaw cycles of the xylem are reported in this (Fig. 6b) and other studies (Hammel 1967; Zimmermann 1983). In previous studies it has been hypothesized that gas bubbles in the xylem water formed during freezing subsequently induces cavitation by expanding under tension during the thawing of the tissues. Additionally, increased tensions during the period of thaw are thought to enhance the probability of the gas bubbles expanding and inducing cavitation (Ewers 1985; Sperry and Sullivan 1992). The loss in hydraulic conductivity in our study was correlated to both shoot water potential and the number of freeze-thaw events (Fig. 6). The lowest shoot water potentials and highest number of freeze-thaw events corresponded to the greatest reduction in hydraulic conductivity during the winter (Fig. 6). These data suggest the development of winter cavitation in *P. contorta* depends on the shoot water potential during thawing of the tissue and the number of freeze-thaw events during the winter.

Davis et al. (1999) found in laboratory measurements that vulnerability to cavitation in the conifer *Abies lasiocarpa* was not enhanced by freezing and thawing. Although the discrepancy between these datasets is not testable with our data, we offer two potential explanations. First, the stems analyzed by Davis et al. (1999) were subjected to only a single freeze-thaw event at a given tension. Possibly small and undetected increases in cavitation occur due to freezing and, over time, multiple freeze-thaw events would have led to larger decreases in hydraulic conductivity. Second, the stems measured by Davis et al. (1999) were placed in a centrifuge and the ends were immersed in water during rotation and freezing. This means that during the thawing process the conduits were in contact with free water at high water potential that would be expected to thaw very near 0°C. Alternatively, conduits within an intact branch in the field may, during a thaw, be in contact with frozen conduits within the stem. A frozen conduit would exhibit a negative water potentials (depending on the pore size, water in frozen wood tissue may be at water potentials less than –100 MPa) and thereby reduce the water potential of the thawing conduit. Therefore, more negative water potentials in adjacent conduits may induce cavitation in the thawing conduit. Consideration of such a mechanism should be the focus of future study.

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