Ultrasound acoustic detection of cavitation events in water conducting elements of Norway spruce wood

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Abstract

Water in living conifer tree stems is transported in the sapwood area which consists of a network of small conducting elements, the tracheids. The liquid water in the conduits is under tension caused by evaporation from the needles, and thus in a metastable state which is maintained by cohesion between water molecules and adhesion of water to the conduit walls. The breakage of the liquid water columns inside the conduits by rapidly expanding gas bubbles is a common phenomenon defined as cavitation. Dehydration induced cavitation occurs when an air bubble is pulled into the lumen of a water-filled conducting element via the pores of the pit membranes from an adjacent conduit already embolised or from intercellular spaces filled with air. As a result, liquid water is replaced by water vapour and later by air. Since the continuity of the liquid water columns is interrupted, the hydraulic conductivity of wood is reduced leading to diminished water supply of the transpiring leaves.

Cavitation events were detected by means of an ultrasound acoustic technique recording emissions in the high-frequency range of 100 kHz to 1 MHz. Emissions are due to elastic oscillations in the cell walls of the conducting element following the cavitation event, because the pressure rises rapidly in the conduit lumen as liquid water at negative pressure is replaced by water vapour very near vacuum pressure. The method is suitable to measure the onset and extent of cavitation in both intact and excised tree stems. Ultrasound acoustic detection combined with measurement of the hydraulic conductivity or the water loss can provide essential information on the resistance of species to stress-induced cavitation.

Key words: conifer wood, hydraulic safety, *Picea abies*, ultrasound acoustic emissions.

1. Introduction

The structure of wood, a naturally occurring biological material, is more complex than the structure of other engineering materials. Norway spruce (*Picea abies* (L.) Karst.) wood consists to more than 90% of a network of small conduits (2 – 5 mm length, 20-40 µm diameter) called tracheids, with most of the remaining cells being ray parenchyma. Tracheids are interconnected by bordered pits consisting of a porous membrane held in a pit chamber (Brändström 2001). Wood fulfills many of the functions required for survival of a tree, including water and nutrient transport, mechanical support and storage of water, carbohydrates and secondary compounds. Variability in anatomical structure and composition of wood within a stem includes within-ring differences known as earlywood and latewood, the radial variations resulting from cambial maturation and sapwood ageing, and the differences associated with different heights (Gartner 1995). The greatest overall cause of wood variation is the presence of juvenile wood and its relative proportion to mature wood. Juvenile wood is directly related to the age of the cambium. Thus, regardless of tree age, a juvenile zone occurs at the top of the tree as well as at its base. It is characterised by shorter cells, larger microfibril angles, more compression wood, different specific gravity and higher lignin content (Zobel and van Buijtenen 1989). Juvenile wood is not much appreciated by the wood industry because of its poor mechanical properties and its shrinkage behaviour, but for tree survival it is of utmost importance due to its high hydraulic safety compared to mature wood (Domec and Gartner 2001).

The liquid water in the tracheids is under tension, caused by evaporation from the needles. The negative pressures in the conduits require high mechanical strength of the cell walls in order to avoid implosion, and hydraulic safety against cavitation of the water column. The breakage of the liquid water columns inside the conduits is defined as cavitation. It is followed by embolisation, the diffusion of dissolved air into the tracheids. Drought stress-induced cavitation occurs when an air bubble is pulled into the lumen of a water-filled conducting element via the pores of the pit membranes from an adjacent conduit already embolised (air-filled) or from intercellular spaces filled with air. Liquid water is replaced by water vapour and later by air. Consequently, the conduit does not conduct water any longer and the hydraulic conductivity of the plant is reduced, thus leading to impairment of water supply of the transpiring needles (Tyree and Zimmermann 2002).

Vulnerability to cavitation can be determined by the construction of vulnerability curves. A vulnerability curve (VC) is a plot of the extent of embolism *versus* the pressure potential in the solution transported in the conduits that induced the embolism or the percentual loss of water content. By establishing VCs the specific water potential thresholds for the onset of cavitation are determined. Methods to induce embolism are dehydration in air, centrifugal force, compressed air applied in a pressure collar and air injection. According to the “air seeding” hypothesis, the “positive pressure needed to blow air through the largest water-filled pores should be the same in magnitude but opposite in sign to that needed to cause embolism (during drought stress)” (Tyree & Sperry 1989). The classic method to measure the extent of embolism is the determination of the native hydraulic conductivity in relation to the hydraulic conductivity after artificially refilling all embolised tracheids. Cavitation events can also be detected by recording ultrasound acoustic emissions (UAEs) in the high-frequency range of 100 kHz to 2 MHz (Tyree and Dixon 1983) (Fig. 1). UAEs are induced by elastic oscillations in the cell wall of the conduits following the cavitation event, because the pressure rises rapidly in the tracheid lumen as liquid water at negative pressure is replaced by water vapour very near vacuum pressure.

This study was a first attempt to find differences in the cavitation behaviour of mature and juvenile wood of Norway spruce by detection of ultrasound acoustic emissions. We also compared the ultrasound acoustic technique to other approaches for constructing VCs.

2. Materials and methods

2.1 Plant material

Wood boles (20 cm length) from freshly felled 50-year-old Norway spruce trees (*Picea abies* (L.) Karst.) were debarked and brought to the laboratory. Outer sapwood samples with a transverse surface of about 1.2 x 1.2 cm were split along the grain with a chisel. Beams with a cross section of 0.8 x 0.8 cm and a length of 13 cm were machined from the samples, with the wood rays parallel to the edges. Tangential and radial faces of the beams were planed on a sliding microtome. During all these steps wood samples were kept wet. The final standard shape of the samples was 0.65 x 0.65 x 13.0 cm.

4-year-old Norway spruces were debarked in the laboratory, and wood samples of 13 cm length (Ø 0.5 – 0.9 cm) were prepared. All samples were soaked in distilled water under vacuum for at least 48 h to refill embolised tracheids.

2.2 VCs based on percent loss of conductivity (PLC) and applied pressure

The specific conductivity (k_s) describes the permeability of a wood segment following Darcy´s Law and is defined as: $k_s = Q * l * A_s^{-1} * \Delta P^{-1}$ [$m^2s^{-1}MPa^{-1}$], where Q is the volume flow rate [m^3s^{-1}], l is the length of the segment [m], A_s is the sapwood cross-sectional area [m^2], and ΔP is the pressure difference between the two ends of the segment [MPa]. Calculated conductivity data were corrected to 20°C to account for changes in fluid viscosity.

The modified Sperry Apparatus designed by Mayr (2002) was used to measure the volume flow rate. Conductivity measurements were carried out under a hydraulic pressure head of 0.008 MPa with distilled, filtered (0.22 µm), and degassed water containing 0.005% (v/v) Micropur (Katadyn Products Inc.) to prevent microbial growth (Sperry et al. 1988). VCs were obtained with methods described by Spicer and Gartner (1998) and Domec and Gartner (2001). These methods involved measuring the PLC on wood samples taken directly from the trunk and saturated in water. After determination of the conductivity at full saturation ($k_{s(i)}$), a pressure (psi) was applied to the lateral sides of the samples, while the transverse ends protruded from a double-ended pressure chamber (PMS Instruments Co., Corvallis, Oregon) to induce cavitation. After pressure treatment samples were wrapped in parafilm® for about 30 min to permit diffusion of air bubbles into tracheids. Thereafter hydraulic conductivity was measured again $k_{s(psi)}$. Initially, the pressure chamber was pressurised to 0.5 MPa, and the pressure was subsequently increased after each conductivity measurement in steps of 0.5 – 1.0 MPa till more than 95% PLC was reached. PLC at a given pressure was calculated using the following equation: PLC [%] = $(k_{s(i)} - k_{s(psi)})/ k_{s(i)} * 100$. Hydraulic VCs were fitted by the least square method based on a sigmoidal function: PLC = $100/(1 + \exp(A(\text{psi} - B)))$, where A indicates the slope of the linear part of the curve and B is the potential at which 50 PLC occurred.

2.3 VCs based on UAE rate and water loss

Monitoring of UAEs was achieved with the µDiSP™ Digital AE system from Physical Acoustics Corporation. Two PCI-2 cards in a portable, 4 channel notebook based chassis allowed simultaneous measurement of 4 samples. 40 dB preamplifiers were used in connection with R15C sensors over a standard frequency range of 50-200 kHz. Data acquisition was performed with a detection threshold of 30 dB, a frequency range between 100 kHz and 1 MHz (Sandford and Grace 1985), and a rate of 5Msamples/second. Parameters recorded were cumulative UAE hits, the rate of UAE hits, the amplitude, the duration, the signal strength and the frequency of UAE hits *versus* time.

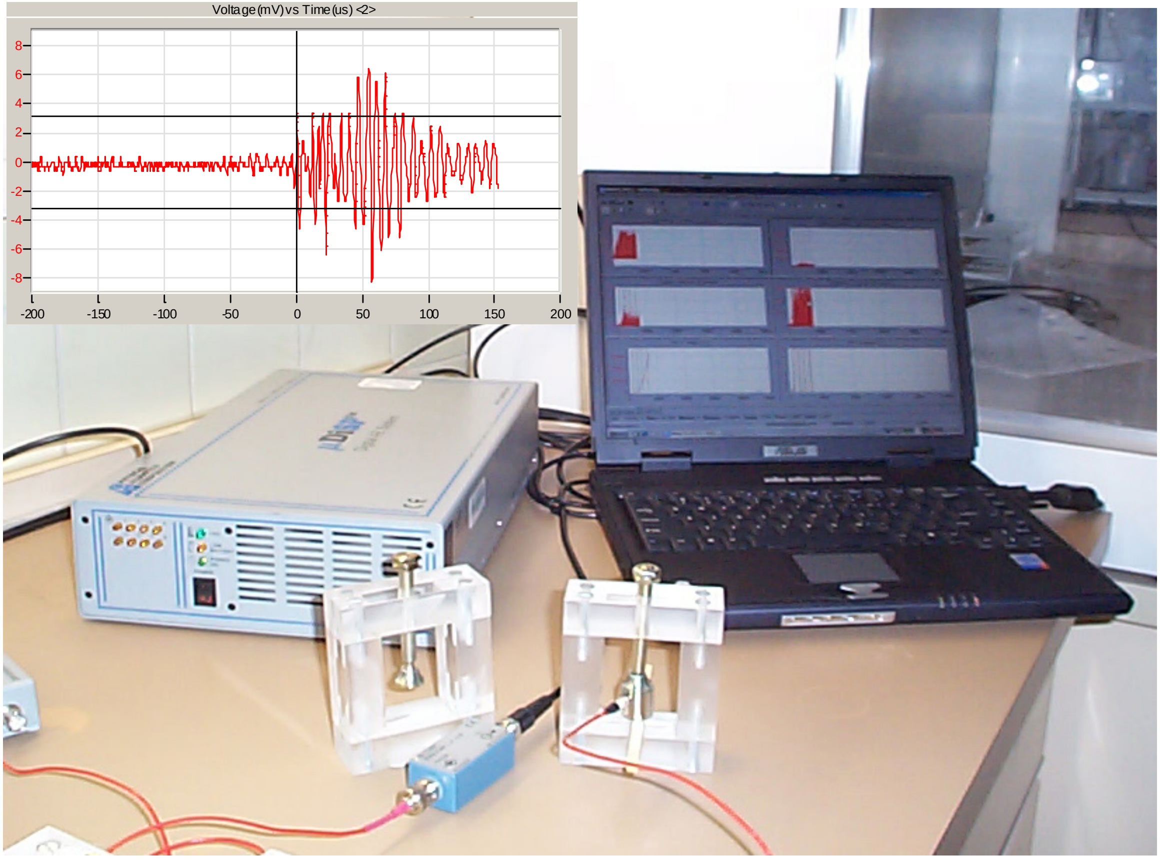


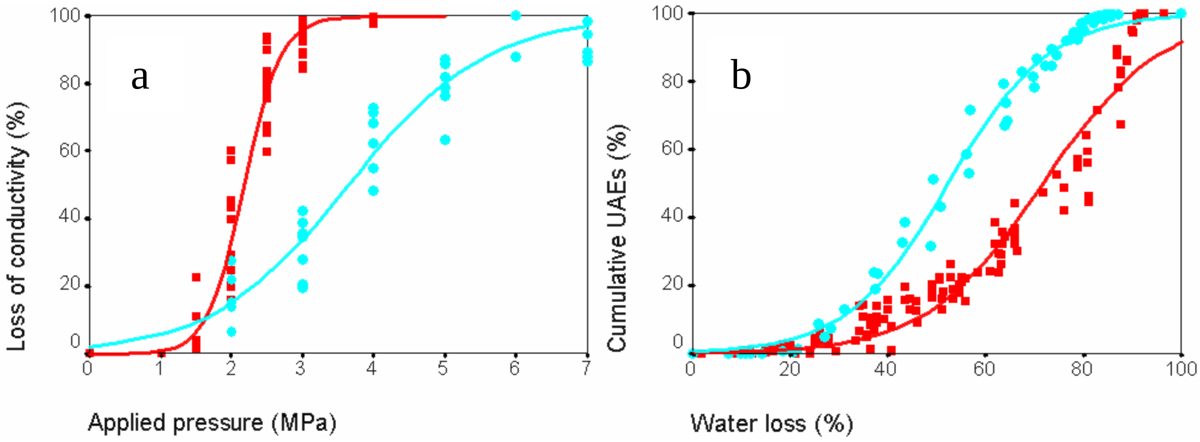
Fig. 1 UAE monitoring equipment, and burst signal of a cavitation event (top left)

UAE sensors were positioned on fully saturated wood samples using an acrylic glass construction (Fig.1). Continuous water loss was detected by placing a sensor plus wood sample on a balance. The weight of the sample was automatically recorded every minute.

Hydraulic VCs were fitted by the least square method based on a sigmoidal function: Cumulative UAEs (%) = 100/(1 + exp(*A*(C - *B*)), where *A* indicates the slope of the linear part of the curve and *B* is the potential at which 50% cumulative UAEs occurred. The water content (C) was calculated as (fresh weight – dry weight) / dry weight; dry weight was obtained by drying wood samples at 103 °C to constant weight. Percent loss of water was calculated by relating the actual water content to the initial water content at full saturation.

3. Results and Discussion

Monitoring hydraulic vulnerability using VCs based on the PLC *versus* applied pressure led to similar results to those described in the literature for juvenile wood (Cochard 1992, Lu et al. 1996); however, no data for mature wood exist for the species studied. 3.7 MPa pressure application resulted in 50% conductivity loss in juvenile wood (a = 1.03; b = - 3.65; R=0.98***), whereas in mature wood only 2.2 MPa were needed to achieve the same effect (a = 3.79; b = -2,16; R=0.98***), indicating that Norway spruce mature wood is much more vulnerable to dehydration (Fig. 2a). Domec and Gartner (2001) were the first to investigate hydraulic vulnerability of mature trunk wood segments and reported also higher hydraulic safety values in juvenile wood compared to mature wood of Douglas fir. The high values of b in mature wood samples indicate wide pit membrane pores of the tracheids and the high numbers of a suggest a narrow range in maximum pore sizes across tracheids (Pammenter and Vander Willigen 1998). In mature wood conductivity loss occurs over a narrower range of applied balance pressures than in juvenile wood (Fig. 2a).



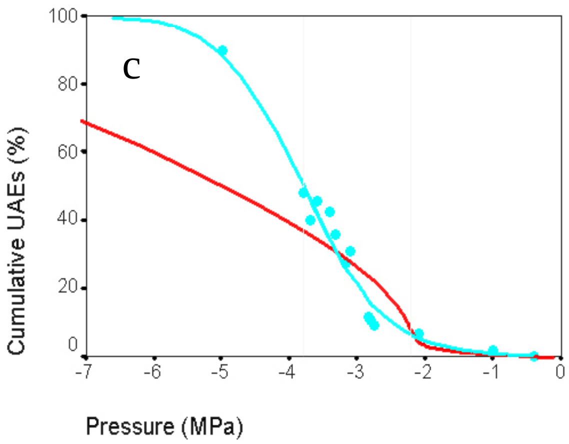


Fig.2 (a) Vulnerability curves based on the loss of conductivity *versus* applied positive pressure in a double ended pressure chamber, blue lines represent juvenile, red lines mature wood; (b) Vulnerability curves based on cumulative UAE hits related to total number of UAE hits *versus* water loss during dehydration at 24°C and 40% rh. (c) Model vulnerability curve for mature wood based on the correlation between water loss and applied pressure as well as UAE rate (red line), and measured VC of a juvenile twig system (A = 1.71; B(50% PCL)= - 3.77 MPa, blue line). Water potential (pressure) measurement was achieved using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA).

Results obtained by recording UAEs *versus* weight loss were difficult to interpret. In juvenile wood 50% cumulative UAEs corresponded to 52.5% water loss (R=0.99***), whereas in mature wood to 71.6% water loss (R=0.98***) (Figs. 2b and 3). In mature wood, the bulk of UAEs occurred only after 50% water loss. These results suggest that mature wood is less vulnerable to cavitation than juvenile wood. Concerning hydraulic conductivity, emissions detected after 50 % water loss cannot be of any physiological relevance for tree survival: we found out that only 36,6 % water loss leads to 50 % loss of hydraulic conductivity in adult wood (R=0.98***).

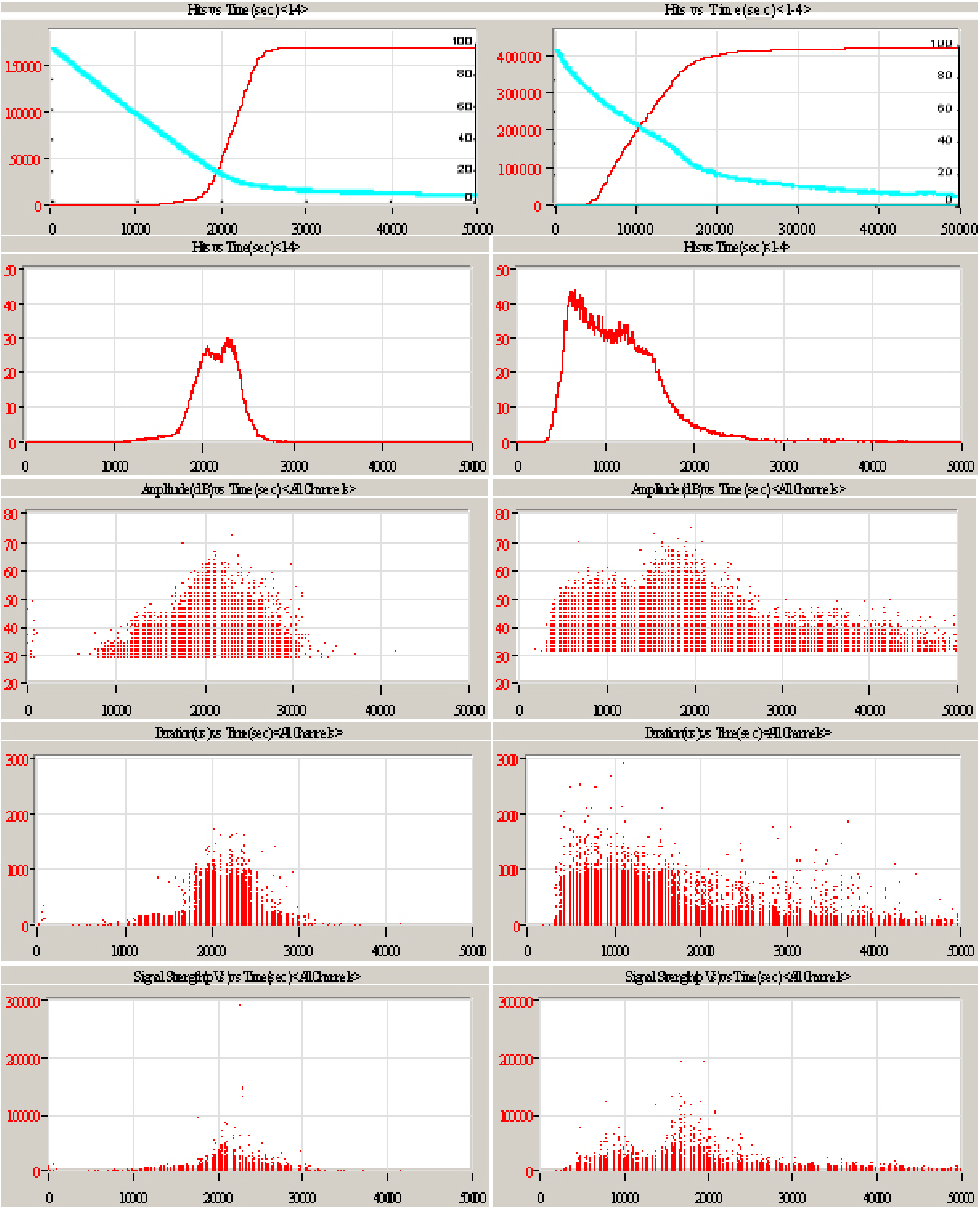


Fig.3 Example of the time course (seconds) of the cumulative UAEs (absolute numbers and % values), the UAE rate/second, the amplitude, the average frequency, and the signal strength of UAE signals during dehydration process at 24 °C and 40 % rh. Left columns represents mature Norway spruce wood samples, right columns juvenile wood samples. The light blue lines in the hits *versus* time windows represent the percent loss of water during the dehydration process (top left, top right).

A model vulnerability curve was calculated for mature wood, based on the strong relationship of the water content to the applied pressure and to the cumulative UAE (%) (cubic regression fit model 0.98*** and 0.99***) (Fig. 2c). VCs based on UAE% *versus* pressure and VCs based on PLC *versus* pressure lead to similar results for 50 PLC (3.77 MPa and 3.65 MPa) in juvenile wood, whereas 50 PLC calculated for adult wood was 5.22 MPa compared to 2.16 MPa, respectively. However, during the range between 2 and 3 MPa mature wood seemed to be more vulnerable to cavitation indicated by the steeper slope of the regression line. The characteristic shape of the relationship between UAE rate and water loss may reflect the varying functions of water within different tissues. After an exponential increase of UAE rate during dehydration of juvenile wood, the signal rate decreased slowly over a relatively long period of time. The signal rate of mature wood was very slow at the beginning, increased rapidly till it reached a plateau of about 25 hits/s and decreased quickly afterwards (Fig. 2). UAEs with low rate at the beginning should be the most important ones concerning their effect on conductivity loss. Vulnerability of a tracheid to embolism is a direct function of its diameter (LoGullo and Salleo 1991, Jackson and Grace 1996). It is well known that mature wood consists of larger tracheids than juvenile wood. The cavitation of a small number of very large (and thus very vulnerable) tracheids may have a dramatic effect on conductivity, since flow rate is proportional to the 4th power of the radius of a capillary. The rapid increase of UAE signals caused by relative a small water loss in juvenile wood may represent the cavitation events of compression wood. Compression wood typically occurs in juvenile trunk wood and branches and is very sensitive to cavitation; its main function is to achieve mechanical safety (Spicer and Gartner 1998, Mayr and Cochard 2003). The successive increase in cavitation events of mature wood after 4 MPa, when PLC reached values below 95% (see Fig 2a), is not easy to interpret. UAE hits recorded after 50 PCL may represent cavitations in wood cells that are not primarily needed for hydraulic conductance but for other functions, such as storage of water. Wood at the base of the trunk is known to have higher water storage capacities than juvenile wood from the top of conifer trees (Domec and Gartner 2001).

The average frequency of UAE hits detected during the dehydration process reached values around 150 kHz in juvenile and mature wood. UAE hits recorded at the beginning of the drying experiments showed shorter duration, smaller amplitude and reduced signal strength (Fig. 3) in mature wood compared to juvenile wood. The main medium of UAE transmission is thought to be cellulose (the main component of tracheid walls) and not the water in the conduits or the cell wall. Norway spruce mature wood contains much more water than juvenile wood because it consists of tracheids with wider lumina, contains less compression wood and is thus less dense. We calculated 220% water content in mature, and about 100% in juvenile wood (water in relation to dry weight). The decrease of the above mentioned traits after extreme water loss should reflect cavitation events of small tracheids less vulnerable to cavitation. Some of the UAE signals also may have been produced by mechanical failure during shrinkage processes.

4. Conclusions

The UAE method is useful for monitoring vulnerability to dehydration in young Norway spruce stems or twigs. For mature wood samples additional measurements are needed, such as the measurement of the hydraulic conductivity in combination with the pressure applied or the water loss. Concerning VCs based on PLC and applied pressure, juvenile wood is less sensitive to dehydration than mature wood. Mature wood showed less sensitivity to cavitation based on VC results constructed from UAEs and loss of water. For mature wood, UAE recording can provide additional information about properties such as the water storage potential of the trunk wood, but many more investigations are needed to establish these relationships firmly.

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