ASSESSMENT OF VULNERABILITY TO CAVITATION IN SMALL WOOD SAMPLES OF *PICEA ABIES* (L. KARST.)

Silviya Wolkerstorfer[1,2], Sabine Rosner[2], and Peter Hietz[2]

[1]Forest Research Institute-BAS, St. Kliment Ochridski 132, 1756 Sofia, Bulgaria; Institute of Botany, University of Natural Resources and Life Sciences, 1180 Vienna, Austria. E-mail: svelichkova.fri@gmail.com

[2]Institute of Botany, University of Natural Resources and Life Sciences, 1180 Vienna, Austria. E-mail: sabine.rosner@boku.ac.at; peter.hietz@boku.ac.at

UDC 630.81

Received: 05 September 2010
Accepted: 21 February 2011

Abstract

The hydraulic effectiveness of the xylem affects tree growth and the ability to survive drought conditions. Under drought stress the water transport system can fail. Trees differ in their capacity to maintain a functional xylem at low water potential, which is described by xylem vulnerability. In this study we present a method for measuring xylem vulnerability curves in small samples. Using tangential sections of Norway spruce (*Picea abies* (L.) Karst.) wood, we quantify the loss in hydraulic conductivity by measuring the number of ultrasound acoustic emissions, which result when water-containing tracheids cavitate. At the same time the water potential of the sample was measured with a thermocouple psychrometer. To test the method we measured vulnerability to cavitation in samples of different wood types. The comparison between juvenile wood and mature earlywood spruce samples indicates that juvenile wood is more resistant to cavitation than mature wood. The anatomy of studied wood samples was also analyzed and was related to xylem functionality.

Key words: hydraulic diameter, juvenile wood, mature wood, *Picea abies*, tracheid thickness to span ratio, xylem vulnerability.

Introduction

Water is lifted from the roots to the leaves of the tree crown under negative pressure (or tension) that is created in the leaf xylem through transpiration. When the tension rises through increasing transpiration demand or reduced supply from the soil, the water columns inside the tracheids may break. The mechanism of dehydration-induced sudden breakage of water columns (cavitation) caused by rapidly expanding air bubbles at the pores of pit membranes between embolized (conduits full with air) and water-filled conduits was demonstrated by Sperry and Tyree (1988). Many embolized conduits severely limit the potential to transport water, the consequence of which can be reduced tree growth and dieback. Xylem dysfunction is generally analyzed by “vulnerability curves”, which represent the increase in the amount of embolism or the consequent reduction in hydraulic conductivity with decreasing water potential in the xylem. Vulnerability curves were used for the estimation of theoretical limits of the water transport in the xylem of several conifers (Tyree and Sperry 1989a). Also, they provide information about the ecophysiological behaviour of the plant (Cochard 1991). Different methods were developed for measuring the embolism rate in xylem samples. The degree of embolism in a stem segment can be estimated by its initial conductivity as a percentage of a maximum obtained after removing the emboli by repeated high pressure flushing (Sperry et al. 1988, Tyree and Sperry 1989b, Vogt 2001, Mayr et al. 2002, Mayr and Cochard 2003), or by using centrifugal force to measure the occurrence of cavitation as a function of negative pressures in xylem (Alder et al. 1997). Xylem conductivity in stem cross sections has been determined by using a dye staining method (Mayr and Cochard 2003, Mayr et al. 2007, Hietz et al. 2008). Only recently, various hydraulic methods for measuring vulnerability were compared and their advantages and potential problems discussed (Hietz et al. 2008). Beside hydraulic methods, xylem embolization has also been studied based on the number of ultrasound acoustic emissions during cavitation. In 1966, Milburn and Johnson found that cavitation events in plants cause a rapid relaxation of tension that produces ultrasound acoustic emission (UAE). First, UAEs were detected using audio (i.e., low-frequency range, <15 kHz) acoustic transducers and amplifiers (earphones), while in the 1980s and later techniques for automatic monitoring of ultrasound acoustic emissions were developed (Tyree and Dixon 1983, Tyree and Sperry 1989b, Beall 2002).

It is well-known that wood properties vary with distance from the pith, e.g., structure and function of the xylem differs between juvenile and mature wood (Domec and Gartner 2003, Rosner et al. 2007, Rosner et al. 2008). Juvenile wood is characterized by tracheids with a smaller diameter and higher wood density compared to mature wood, leading to a lower hydraulic conductivity and vulnerability to cavitation (Domec and Gartner 2001, Rosner et al .2006).

The main objective of the current study is to develop a method for analyzing xylem vulnerability in small stem samples (*Picea abies* (L.) Karst.) using an approach based on ultrasound acoustic emissions, and to validate the method as we relate the hydraulic vulnerability to the anatomical properties of the wood.

Material and Methods

Sample preparation

Juvenile and mature wood samples were taken from 25-year-old Norway spruce (*Picea abies*) growing in southern Sweden (latitude 56°67′, longitude 13°07′, 60 m above sea level). The sample material was harvested during a wet period in the middle of June, 2008, when trees did not suffer from drought stress. Trunk wood samples were taken immediately after felling, juvenile wood from the top of the tree (third internode) from the beginning of the crown and mature wood at a height of 1 m above the ground. Wood boles were debarked, split along the grain, and the outer 2 cm of the split sapwood part were transported to BOKU, Vienna (Austria) in plastic bags with fresh water containing 0.01% Micropur (Katadyn Producte AG, Walliseellen, Switzerland), preventing microbial growth. Samples were thereafter stored at -18°C. From the sapwood pieces, tangential rectangle samples (1.1 cm x 0.7 cm x and from 0.33 to 0.4 mm thick) were isolated under a binocular. From the mature sapwood samples, earlywood with homogenous lumen diameters, which can be found between springwood (first formed earlywood cell rows) and latewood, was isolated. Young juvenile wood (age < 3 years) shows density variations and a less distinct latewood zone. Specimens were split from juvenile wood regions with quite uniform wood structure avoiding compression wood. Afterwards specimens were soaked in distilled water containing 0.01% Micropur, and kept under vacuum for several hours until air bubbles stopped coming out from the sample, which indicates full saturation.

Measurements of wood split samples from spruce trees were made based on the modified method employed by Kikuta et al. (2003). For this purpose a new chamber for measuring the small wood samples was designed. The chamber has a cylindrical shape where in the middle a removable metal grid is situated onto which the wood section was placed. The sample was on one side directly attached to an R15C transducer connected to a μDiSP™ Digital acoustic emission system from the Physical Acoustic Corporation (Princeton Junction, NJ, USA), which recorded the UAEs over a standard frequency range of 50–200 kHz. A thermocouple psychrometer was positioned on the opposite wood surface in order to measure the water potential. The psychrometer was placed inside the chamber where vapor pressure equilibrium between water potential in the wood sample and vapor pressure in the air are in dynamic equilibrium. At the beginning of our experiments, when the samples were saturated with water, the relative humidity of the vapor phase was close to 100% and the samples had a water potential slightly below 0 MPa. As the wood dried the water potential decreased and cavitations were recorded in parallel. The acoustic emissions recorded during drying the samples were calculated in percent of the total number of emissions (%UAE = 100 $UAE^{drying\ sample}/UAE^{total}$).

Anatomical investigations

Transverse sections were made from each of the wood samples that had been used to produce vulnerability curves. Three 30 μm thick sections were made with a sliding microtome, stained in methylene blue and mounted in Entellan (Merck, Darmstadt, Germany). Of each slice three pictures were made randomly over the whole radial length of the sample with a Leica DM4000M microscope interfaced with a digital camera (Leica Microsystems Wetzlar GmbH, Germany). The tracheid lumina and cell wall thickness were measured with SigmaScan Pro 5 (Systat Software Inc., Chicago, USA). First, the mean hydraulic diameter $D = \Sigma d^5 . (\Sigma d^4)^{-1}$ was calculated, where d is the individual conduit diameter. For each sample separately, six rows of tracheids were randomly selected and the diameter of all the tracheids was measured in radial direction. To characterise the conduit wall reinforcement against collapse from bending we measured the "double wall thickness to span ratio" $(t.b^{-1})^2$. The $(t.b^{-1})^2$ ratio was calculated for each sample, where only the cells with a diameter of within 10% of D were considered (Domec et al. 2009). The double cell wall thickness of adjacent conduits (t) and the conduit lumen (span, b) were measured on 26–40 tracheid pairs.

Results and Discussion

The main result we can draw from our experiments is that juvenile wood was more resistant to cavitation than mature early wood. The water potential required to achieve a 50% loss of conductivity was -2.82 MPa for juvenile wood and -1.47 MPa for mature wood (Fig. 1).

The study of wood anatomy showed that juvenile wood has a smaller mean hydraulic diameter and a higher tracheid wall to span ratio $(t.b^{-1})^2$ than mature earlywood (Tab. 1, Fig. 2).

The trade-off between vulnerability to cavitation and hydraulic conductivity observed in our study for mature and juvenile wood (Fig. 1, Tab. 1) confirmed the results obtained by other authors (Domec and Gartner 2001, Rosner et al. 2006). Vulnerability to embolism is

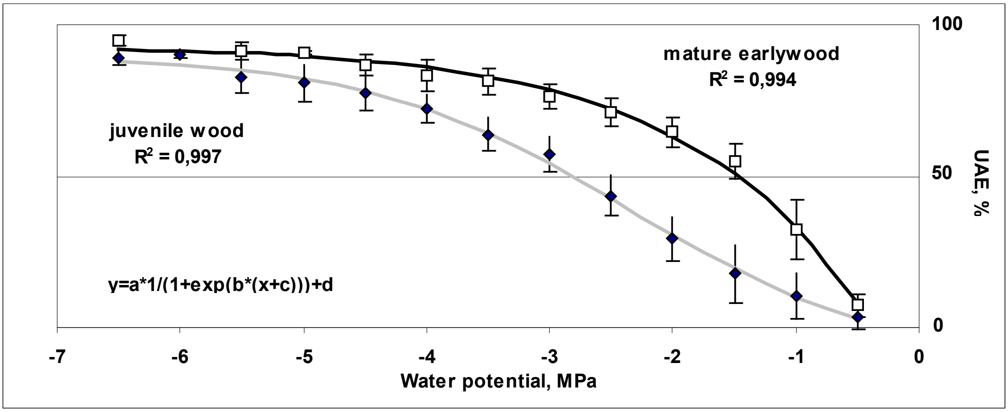

Fig. 1. Vulnerability curves for small stem segments (1 cm x 0.7 cm) of mature earlywood and juvenile wood taken from *Picea abies*. Percent ultrasound acoustic emissions (UAE) are plotted versus water potential. Each plot is the mean of the percent UAE measured in intervals of 0.5 MPa of water potential on 5 (juvenile) and 4 (mature earlywood) stem segments. Error bars show standard errors. The solid lines resulted from sigmoidal regression and are described by the given equation. The same function was used to interpolate the water potential at 50% UAE.

known to be influenced by the structure of the xylem (Hacke et al. 2001, Domec and Gartner 2002) and pit pore characteristics (Domec et al. 2006). Larger conduits are often more susceptible to cavitation (Hargratte et al. 1994). According to the air-seeding hypothesis (Zimmermann 1983) air enters the lumen of water-filled vessels through pores in the pit membrane of the walls of embolized conduits. Consequences thereof are cavitation and the following embolism. Wider conduits such as the tracheids in mature earlywood have larger pores in their pit membranes, allowing easier air penetration (Tyree and Sperry 1989a). These elements therefore are more conductive than juvenile wood. Additionally, thicker cell walls in juvenile wood are associated with a thicker pit

Table 1. Mean hydraulic diameter (D) and tracheid thickness to span ratio $(t.b^{-1})^2$ in Picea abies juvenile wood and mature earlywood stem sections used above for producing vulnerability curves. Means (±SE) are shown.

	D, μm	$(t.b^{-1})^2$
Juvenile wood	25.63 ± 1.40	0.046 ± 0.012
Mature early wood	31.30 ± 2.05	0.037 ± 0.010

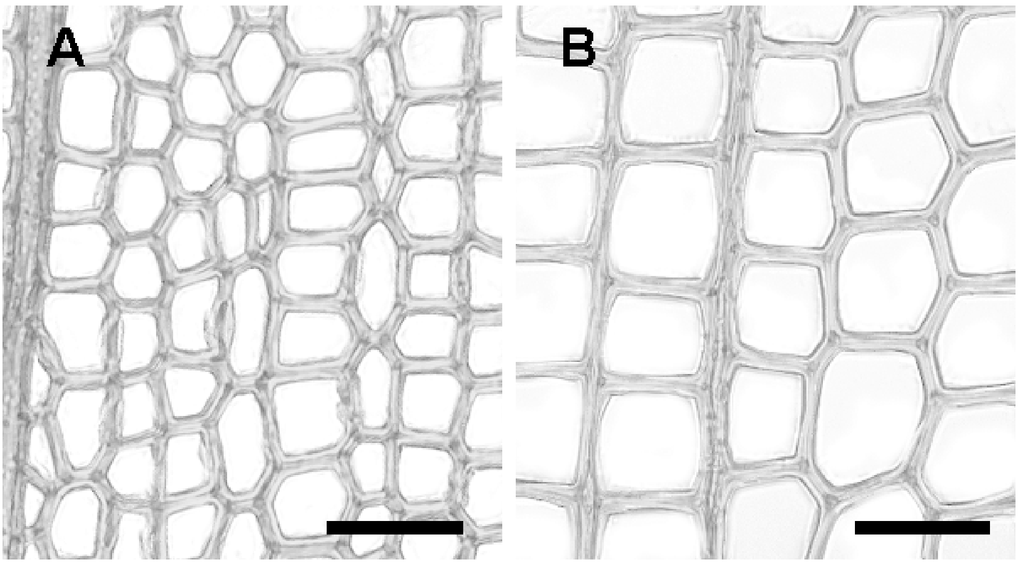

Fig. 2. Transmission light microscope pictures made on transverse juvenile (A) and mature (B) stem *Picea abies* sections showing tracheid lumen and cell wall thickness. The reference bar is 30 μm.

chamber depth, making this wood more resistant to air seeding (Domec and Gartner 2001). On the other hand, the measured increase in cell lumen diameter relative to cell wall thickness in mature early wood is consistent with the higher hydraulic vulnerability of mature early wood (Tab. 1).

Conduits carrying water over long distances under negative pressure need reinforced and lignified cell walls to prevent implosion (Hacke and Sperry 2001). The safety factor against implosion caused by negative pressure, given by tracheid thickness to span $(t.b^{-1})^2$ ratio, was seen to be proportional to resistance to cavitation in the studied wood samples. The decrease of the $(t.b^{-1})^2$ ratio from juvenile to mature wood as vulnerability to cavitation increases has also been described for other conifers species (Domec and Gartner 2001).

Our study has shown that the developed method can be used for simultaneous measurements of the percent loss of conductivity and the corresponding water potential in small wood samples. We confirmed a relation between cell anatomy and cavitation resistance: juvenile wood had a higher $(t.b^{-1})^2$ ratio compared to mature earlywood and correspondingly a higher resistance to cavitation. Furthermore, our results support the conclusion made in previous studies that juvenile wood is more resistant to cavitation than mature early wood.

Acknowledgments

The first author was supported through an "Ernst Mach" grant by the OeAD. The work was also partly supported by the FWF Project T304-B16.

References

Alder N. N., Pockman W. T., Sperry J. S., Nuismer S. 1997. Use of centrifugal force in the study of xylem cavitation. Journal of Experimental Botany, 48: 665–674.

Beall F. C. 2002. Overview of the use of ultrasonic technologies in research on wood properties Wood Science and Technology, 36: 197–212.

Cochard H. 1991. Vulnerability of several conifers to air embolism. Tree Physiology, 11: 73–83.

Domec J.-C., Gartner B. L. 2001. Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees, 15: 204–214.

Domec J.-C., Gartner B. L. 2002. Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas fir wood structure. Tree Physiology, 22: 91–104.

Domec J.-C., Gartner B. L. 2003. Relationship between growth rates and xylem hydraulic characteristics in young, mature, and old-growth Ponderosa pine trees. Plant, Cell and Environment, 26: 471–483.

Domec J.-C., Lachenbruch B., Meinzer C. F. 2006. Bordered pit structure and function determine spatial patterns of air-seeding thresholds in xylem of Douglas-fir (Pseudotsuga menziesii; Pinaceae) trees. American Journal of Botany, 93: 1588–1600.

Domec J.-C., Warren J. M., Meinzer F. C., Lachenbruch B. 2009. Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. JAWA Journal, 30 (2): 100–120.

Hargrave K. R., Kolb K. J., Ewers F. W., Davis S. D. 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera*

Greene (Labiatae). New Phytologist, 126: 695–705.

Hacke U. G., Sperry J. S., Pockman W. T., Davis S. D., McCulloh K. A. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia, 126: 457–461.

Hacke U. G., Sperry J. S. 2001. Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics, 4: 97–115.

Hietz P., Rosner S., Sorz J., Mayr S. 2008. Comparison of methods to quantify loss of hydraulic conductivity in Norway spruce. Annals of Forest Science, 65 (5): 502–507.

Kikuta S. B., Hietz P., Richter H. 2003. Vulnerability curves from conifer sapwood sections exposed over solution with known water potential. Journal of Experimental Botany, 54 (390): 2149–2155.

Mayr S., Wolfschwenger M., Bauer H. 2002. Water drought induced embolism in Norway spruce (*Picea abies*) at the Alpine timberline. Physiologia Plantarum, 115: 74–80.

Mayr S., Cochard H. 2003. A new method for vulnerability analysis of small xylem areas reveals that compression wood of Norway spruce has lower hydraulic safety than opposite wood. Plant, Cell and Environment, 26: 1365–1371.

Mayr S., Cochard H., Améglio T., Kikuta S. B. 2007. Embolism formation during freezing in the wood of *Picea abies*. Plant Physiology, 143: 60–67.

Milburn J. A., Johnson R. P. C. 1966. The conduction of sap. II. Detection of vibrations produced by sap cavitation in *Ricinus* xylem. Planta, 66: 43–52.

Rosner S., Klein A., Wimmer R., Karlsson B. 2006. Extraction of features from ultrasound acoustic emissions: a tool to assess the hydraulic vulnerability of Norway spruce trunkwood? New Phitologist, 171: 105–116.

Rosner S., Klein A., Müller U., Karlsson B. 2007. Hydraulic and mechanical properties of young Norway spruce clones related to growth and wood structure. Tree Physiology, 27: 1165–1178.

Rosner S., Klein A., Müller U., Karlsson B. 2008. Tradeoffs between hydraulic and mechanical stress response of mature Norway spruce trunkwood. Tree Physiology, 28: 1179–1188.

Sperry J. S., Tyree M. T. 1988. Mechanism of water stress-induced xylem embolism. Plant Physiology, 88: 581–587.

Sperry J. S., Donnelly J. R., Tyree M. T. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell and Environment, 11: 35–40.

Tyree M. T., Dixon M. A. 1983. Cavitation events in *Thuja occidentalis* L.? Ultrasonic acoustic emissions from the sapwood can be measured. Plant Physiology, 72: 1094–1099.

Tyree M. T., Sperry J. S. 1989a. Vulnerability of xylem to cavitation and embolism. Annual Review of Plant Physiology and Molecular Biology, 40: 19–38.

Tyree M. T., Sperry J. S. 1989b. Characterization and propagation of acoustic emission signals in woody plants: towards an improved acoustic emission counter. Plant, Cell and Environment, 12: 371–382.

Vogt K. U. 2001. Hydraulic vulnerability, vessel refilling, and seasonal courses of stem water potential of *Sorbus aucuparia* L. and *Sambucus nigra* L. Journal of Experimental Botany, 52 (360): 1527–1536.

Zimmermann M. H. 1983. Xylem structure and the ascent of sap. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag. 143 p.