Journal of Experimental Botany Advance Access published April 12, 2013

Journal of Experimental Botany
doi:10.1093/jxb/ert090
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Investigating xylem embolism formation, refilling and water storage in tree trunks using frequency domain reflectometry

Guang-You Hao[1,2,*,†], James K. Wheeler[2,*], N. Michele Holbrook[2] and Guillermo Goldstein[3,4]

[1] The Arnold Arboretum of Harvard University, Boston, MA 02131, USA
[2] Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
[3] Department of Biology, University of Miami, Coral Gables, FL 33124, USA
[4] Laboratorio de Ecología Funcional (CONICET), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Nuñez, Buenos Aires, Argentina

* These authors contributed equally to the manuscript.
† To whom correspondence should be addressed. E-mail: guangyouhao01@fas.harvard.edu

Received 12 January 2013; Revised 13 February 2013; Accepted 4 March 2013

Abstract

Trunks of large trees play an important role in whole-plant water balance but technical difficulties have limited most hydraulic research to small stems, leaves, and roots. To investigate the dynamics of water-related processes in tree trunks, such as winter embolism refilling, xylem hydraulic vulnerability, and water storage, volumetric water content (VWC) in the main stem was monitored continuously using frequency domain moisture sensors in adult *Betula papyrifera* trees from early spring through the beginning of winter. An air injection technique was developed to estimate hydraulic vulnerability of the trunk xylem. Trunk VWC increased in early spring and again in autumn, concurrently with root pressure during both seasons. Diurnal fluctuations and a gradual decrease in trunk VWC through the growing season were observed, which, in combination with VWC increase after significant rainfall events and depletion during periods of high water demand, indicate the importance of stem water storage in both short- and long-term water balance. Comparisons between the trunk air injection results and conventional branch hydraulic vulnerability curves showed no evidence of 'vulnerability segmentation' between the main stem and small branches in *B. papyrifera*. Measurements of VWC following air injection, together with evidence from air injection and xylem dye perfusion, indicate that embolized vessels can be refilled by active root pressure but not in the absence of root pressure. The precise, continuous, and non-destructive measurement of wood water content using frequency domain sensors provides an ideal way to probe many hydraulic processes in large tree trunks that are otherwise difficult to investigate.

Key words: Cavitation, freeze–thaw stress, root pressure, water relations, water storage, winter embolism refilling, xylem hydraulic vulnerability.

Introduction

The tree trunk, with both long-distance water transport and storage functions, plays an important role in whole-plant water relations (Goldstein *et al.*, 1998; James *et al.*, 2003). However, due to technical difficulties, most studies on tree hydraulic architecture are limited to peripheral organs, such as roots, terminal branches, and leaves, while studies of trunks of adult trees are largely absent. A long-held hypothesis regarding the spatial patterns of plant hydraulics along the whole-plant water transport pathway is known as hydraulic segmentation (Zimmermann, 1978, 1983). According to this paradigm, plants should have structural features that prioritize the water flow continuity in the main axis to the terminal apex. Tyree and Ewers (1991) advanced Zimmermann's segmentation theory by adding the 'vulnerability segmentation' hypothesis. They argued that both higher hydraulic conductivity and stronger resistance to drought-induced cavitation

© The Author(2) [2013].
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

might be necessary for a tree to maintain an intact water column in its main stem; however, few studies have examined the cavitation vulnerability of big stems or trunks of large trees. In the present study, an air injection technique was developed to estimate the xylem vulnerability to air seeding in main stems of adult *Betula papyrifera* Marsh, which is a deciduous tree with diffusely distributed vessels in the xylem.

Besides drought, the freeze–thaw event is another important factor that can induce substantial cavitation in temperate trees during cold seasons (Magnani and Borghetti, 1995; Jaquish and Ewers, 2001; Améglio *et al*., 2002). When the xylem sap freezes, dissolved gases come out of solution and form bubbles that can nucleate cavitation upon thawing when tensions are re-established (Hammel, 1967; Zimmermann, 1983). According to Laplace's law, bubbles smaller than a critical size do not expand and re-dissolve easily, while larger bubbles tend to expand under tension (Oertli, 1971; Vogel, 1988; Yang and Tyree, 1992). Therefore, larger-volume xylem conduits are more vulnerable to freeze–thaw induced embolism than smaller-volume ones because they contain more dissolved gas and form larger bubbles during freezing (Ewers, 1985; Sperry and Sullivan, 1992). Embolism development over the winter caused by freeze–thaw cycles can be extensive in branches of temperate trees, in some cases eliminating all hydraulic conductivity by the end of the winter (e.g. Cochard and Tyree, 1990; Sperry and Sullivan, 1992; Wang *et al*., 1992). Tree trunks with larger xylem conduits compared to those of smaller branches may also develop extensive cavitation over the winter, although this has been documented only in saplings (Sperry *et al*., 1988).

Cavitation does not pose a serious problem during winter, but may impair growth during the following season if newly produced xylem alone is insufficient to supply water to the crown. In many species, mechanisms have evolved for restoring hydraulic conductivity in the spring by refilling the embolized vessels using positive root pressure (Sperry *et al*., 1987, 1994; Hacke and Sauter, 1996; Améglio *et al*., 2002; Cobb *et al*., 2007). Although root pressure has been well documented in many temperate tree and vines species for winter embolism refilling, the dynamic change of xylem water status during the repair process has not been examined, especially in the main stems of adult trees.

The root pressure and trunk volumetric water content (VWC) were measured during the spring throughout the embolism refilling process in *B. papyrifera*. To further investigate the dynamics of trunk water status pertaining to whole-plant water balance, VWC was measured continuously through the summer season to the end of the year until freezing occurred in the xylem. The non-destructive and continuous *in situ* measurements of trunk water status permitted a detailed investigation of a series of important questions regarding plant water relations, such as the role of trunk water storage in short- (diurnal) and long-term (seasonal) water balances, and embolism formation and repair.

Both the trunk xylem hydraulic vulnerability estimation technique and the continuous monitoring of trunk water status used in the present study are based on the novel use of frequency domain reflectometry (FDR) moisture sensors for the *in situ* non-destructive measurement of wood VWC in functioning trees. Moisture sensors based on the FDR technique have been widely used in estimating soil water content (Czarnomski *et al*., 2005), but have not been used in wood of living trees. The FDR sensor, with high precision, high temporal resolution, and convenient installation configuration, is a potentially ideal way to measure stem water content in trunks accurately and continuously, providing insight into both short- and long-term hydraulic related physiological processes. Specifically, in the present study the simultaneous and continuous measurements of root pressure and trunk water content in the spring provide information regarding the dynamics of winter cavitation refilling in the main stems of large trees.

Materials and methods

Study site and plant materials

The study was conducted at Harvard Forest (42° 32' N 72° 11' W), Petersham, Massachusetts, USA. The study sites are mixed secondary deciduous forest stands, dominated by red oak (*Quercus rubra* L.) and red maple (*Acer rubrum* L.), with paper birch (*B. papyrifera* Marsh.) commonly found. Average annual precipitation at this site is *c.*1000 mm and is distributed fairly evenly throughout the year. Annual mean temperature is 6.5 °C, with the highest and lowest monthly mean temperatures occur in July (20 °C) and January (–7 °C), respectively. Adult *B. papyrifera* trees with diameter at breast height ranging from 14.1 to 26.3 cm were used for the present study.

Volumetric water content

Moisture sensors based on FDR technology (model GS3, Decagon Devices, Pullman, WA, USA) were installed in the trunks of adult *B. papyrifera* trees at breast height. Three holes of 5.5 cm in depth spaced 2.54 cm apart were drilled radially into the trunk using an electric drill, with a drill bit of 3.26 mm in diameter. A customized drill guide with three holes of the same diameter was used to facilitate the drilling. After inserting the three prongs (5.5 cm in length, 3.26 mm in diameter, and 2.54 cm apart) of the sensor into the drilled holes, the moisture sensors were gently hammered until the prongs were completely within the tree (Supplementary Fig. S1, available at *JXB* online). The gaps between the sensor overmould and the tree were sealed with silicone caulk to prevent infiltration of water from precipitation. The whole sensor was insulated with foam and aluminium foil to avoid heating by direct sun light. In addition to VWC, trunk temperature was measured by the thermocouples embedded in the sensor overmould. Data were logged using EM50 data loggers (Decagon Devices) every 5 minutes for 24 hours a day. Three sensors were installed in three trees (numbered 1, 2, and 3) in early spring (March) of 2012 and measurements were made continuously until the end of the year. The FDR sensor calibration was conducted in the laboratory using wood of *B. papyrifera* and the results showed that the sensor VWC output based on the factory calibration matches well with VWC calculated by a gravitational method (Supplementary Fig. S2). The factory calibration was thus used in the present study.

Root pressure

Hydrostatic pressures in trunks of the same three trees were measured using electronic pressure transducers (PX26–015GV, Omega Engineering, Stamford, CT, USA). Two holes of 1.59 mm in diameter and *c.*2 cm in depth were drilled near the base of a tree trunk and hypodermic needles of the same outer diameter with bent tips (to avoid clogging by wood debris) were inserted to the sapwood and glued in place with epoxy. The pressure transducers were connected to the hypodermic needles through a short piece

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

of non-elastic water-filled tubing (Bev-A-Line IV, Cole Parmer Instrument, Chicago, IL, USA). The output voltage of the pressure transducers was logged at 5-min intervals with a CR10X data logger (Campbell Scientific, Logan, UT, USA). Every 2–3 weeks, transducers were moved to freshly drilled holes to avoid gradual clogging of xylem. Measurements of root pressure were made from March to June and from September to November 2012.

The pressure transducers were calibrated in laboratory prior to and after the field measurements and no significant drift was found. Briefly, transducers were connected with a high-precision digital pressure gauge (DPG1000AD) and were connected to a high-pressure nitrogen tank through a regulator. The pressure applied to the transducer was increased in a stepwise manner from 0 to *c.*138 kPa with an interval of 1 psi (*c.*6.89 kPa) and the output data were recorded using a CR10X data logger. Linear regressions between voltage outputs and applied pressures were used to calculate the root pressures.

Vulnerability to cavitation in tree trunks and terminal branches

To determine whether the early spring stem water content increase is directly related to the refilling of winter-embolized vessels, an air injection technique was developed to induce embolism in the sapwood where the moisture sensors were located. A high-pressure-resistant PEEK tubing (MicroSolv, Eatontown, NJ, USA) was inserted into a freshly drilled hole in the tree trunk (*c.*1.59 mm in diameter and *c.*5 cm in depth) 2 cm above the installed FDR sensor and held in place with a customized tubing holder, which was tied to the trunk using a ratcheting tie-down (Supplementary Fig. S1). The tubing was connected to a digital pressure gauge (DPG1000AD) and a high-pressure nitrogen tank through a regulator. An o-ring was placed around the PEEK tubing between the tree bark and the tubing holder to make a tight seal. The injection pressure was increased in a stepwise manner from 0 to 3.5 MPa at intervals of about 0.3 MPa. The pressure for air injection was kept constant at each level for 15–25 min until VWC (FDR sensor readings were continuously monitored with a computer) reached a steady state. Air injection was conducted on four trees (trees 2 and 3 plus two trees not used for long-term VWC monitoring) in mid-April of 2012 before leaf flush when VWC had increased to a relatively stable level. To test whether embolism can refill in the absence of root pressure, injection was also conducted on another four trees (trees 5–7 and another tree not used for long-term VWC monitoring) at a different site in early June. Trees 1 and 4, which were not air-injected, were used as controls for spring and summer measurements, respectively. Continuous measurements of trunk water content were recorded for trees 4–6 (tree 7 was cut down after air injection) from 25 May 2012 to the end of the year.

Conventional hydraulic vulnerability curves were constructed using centrifugal force method on five small stem segments from different trees with diameters ranging from 0.5 to 0.8 cm (Alder *et al.*, 1997). Briefly, vulnerability to drought-induced cavitation was measured as the decrease of hydraulic conductivity (K_h) in response to a stepwise increase in xylem tension generated by spinning a 14.2-cm stem segment using a Sorvall high-speed centrifuge (RC-5C Plus, Du Pont Instruments, Wilmington, DE, USA) with a custom rotor (Alder *et al.*, 1997). The percentage loss of conductivity (PLC) following each spinning was calculated as $100[(K_{max}–K_h)/K_{max}]$, where K_h is the hydraulic conductivity after each spinning and K_{max} is the maximum conductivity measured on flushed segments.

Dye perfusion

In June, one tree was cut down 0.9 m above the ground after the completion of air injection and a trunk segment *c.*60 cm (with the injection point in the middle) of the trunk was cut off. The segment was kept under water in a cooler and transported to the laboratory. Using an electric saw, 5 cm was removed from both ends and the remaining segment was connected to a soft polythene tube (modified from a ziplock bag) with a diameter of *c.*15 cm. Parafilm was used to ensure a tight seal between the tube and the segment. The segment was placed vertically and the reservoir created by the polythene tube on top of the stem segment was filled with 2 l of filtered 0.1% toluidine blue solution. On the second day, after all the staining solution in the reservoir had flowed through the trunk segment, it was cut transversely into two pieces in the middle to evaluate the effect of air injection on xylem hydraulic conductivity.

Wood anatomy

Small pieces of trunk sapwood were taken using a chisel from four trees at breast height and transverse sections of these wood samples were prepared using a sliding microtome (Reichert, Vienna, Austria). Wood cross-sections were also made on small branches with diameters ranging 0.5–0.8 cm. These cross-sections were stained in a 0.1% toluidine blue solution and images were taken at magnification ×40 (trunk wood) or ×100 (branch) using a digital camera (Axiocam HRc, Carl Zeiss, Jena, Germany) mounted on a light microscope (Olympus BH-2, Olympus, Tokyo, Japan). Vessel diameters were calculated using ImageJ software (US National Institutes of Health, Bethesda, MD) based on individual lumen areas assuming circular shapes.

Results

Xylem water recharge under root pressure in early spring

With the occurrence of root pressure in mid-March 2012, trunk VWC quickly increased to a substantially higher level, which was on average 0.08 m^3 m^{-3} higher than the initial average value (Fig. 1A, B). Before wood VWC reached a stable level, it exhibited regular diurnal fluctuations with higher values observed at midday, which corresponded very well with the diurnal fluctuations in root pressure. After a maximum VWC level was reached, the diurnal fluctuation became negligible although root pressure continued to show a diurnal pattern of variation (Fig. 1 and Supplementary Fig. S3A, B).

The high resolution of the GS3 moisture sensor made it possible to measure subtle changes in wood water contents. However, when the water in the xylem froze, the output of the sensor did not reflect the actual water content (Fig. 1, see also Fig. 3) due to the large difference in dielectric constant between liquid and solid water (80 vs. 3.2 for water and ice at 0 °C), which is the basis for calculating VWC (Kizito *et al.*, 2008). Thus in a few cases, such as on 27 March, the dramatic decrease in VWC readings from the sensor resulted from freezing rather than a change of absolute water content (including ice) in the trunk wood (Fig. 1A). Likewise, the very high readings of pressure transducers during the few days with below freezing temperatures did not imply high root pressure but rather a pressure increase caused by the volume expansion of water during freezing in the tubes connecting the xylem and inside the transducer chambers (Fig. 1B). Aside from these few days when freezing occurred, both VWC and root pressure measurements were very reliable.

Short-term and seasonal variations in trunk water content

After full leaf expansion, diurnal fluctuations in trunk VWC reoccurred but with a different pattern compared to that

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

found before and during xylem refilling; on sunny days, the maximum VWC occurred at night and the minimum values occurred around midday, which was consistent with the diurnal pattern of evaporative demand (Supplementary Fig. S3C). Significant increases in trunk VWC during days with significant rainfall and gradual decreases thereafter were observed (Fig. 2A and Supplementary Figs. S4 and S5). During a heat wave in late June, VWC of the trunk decreased substantially indicating the use of internal water storage in the trunk to buffer high canopy water demands due to elevated air saturation deficits (Fig. 2B and Supplementary Figs. S4 and S5).

After leaf expansion (around 30 April), root pressure diminished and the overall VWC in the trunk started to decrease gradually until the end of July (Fig. 3A, B and Supplementary Figs. S4 and S5). Trunk VWC started to increase again in the autumn and more noticeably with the resumption of root pressure in October and early November (Fig. 3A, B and Supplementary Figs. S4 and S5). Note that the VWC increase in the autumn occurred over a period of about 1 month and was not as dramatic as that occurred in the early spring, which rapidly reached a maximum level over roughly 2 weeks from the initiation of root pressure (Fig. 1A and Supplementary Figs. S4 and S5).

Cavitation induced by air injection and recovery under root pressure

The injection of air into the tree trunk close to the FDR sensor resulted in a pattern of VWC reduction similar to the decline in hydraulic conductance observed for stems subjected to air injection or centrifugation. When the change of VWC in response to each air injection was expressed relative to the total VWC change observed at the highest applied pressure, the curves are similar to hydraulic vulnerability curves measured on small stem segments of the same species (Fig. 4A). At a pressure of 1.6 MPa, a 50% of the relative VWC decrease was obtained which was not significantly different from the centrifugal force causing 50% loss of hydraulic conductivity

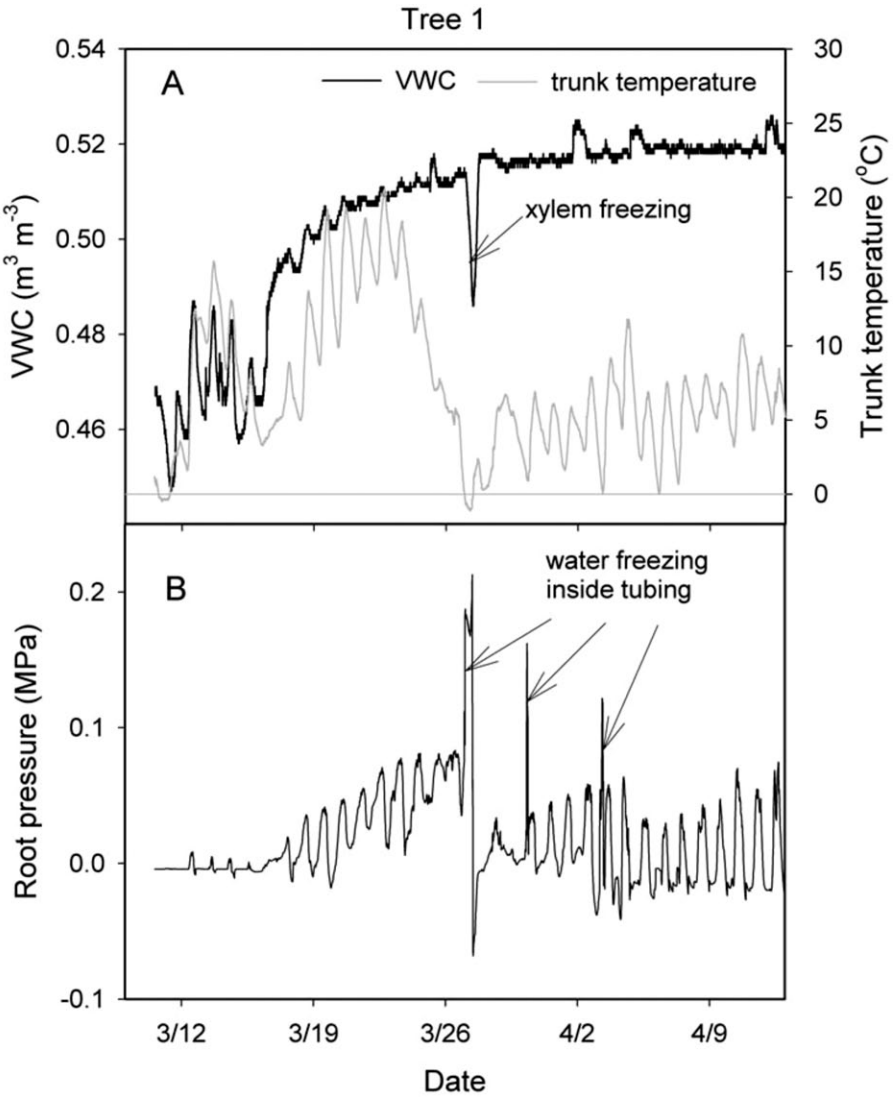

Fig. 1. Diurnal fluctuations in the trunk of a representative *B. papyrifera* tree during early spring in (A) volumetric water content (VWC) and root (xylem) pressure, (B) measured in the same trunk. The horizontal line in (A) shows tree trunk temperature of 0 °C.

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

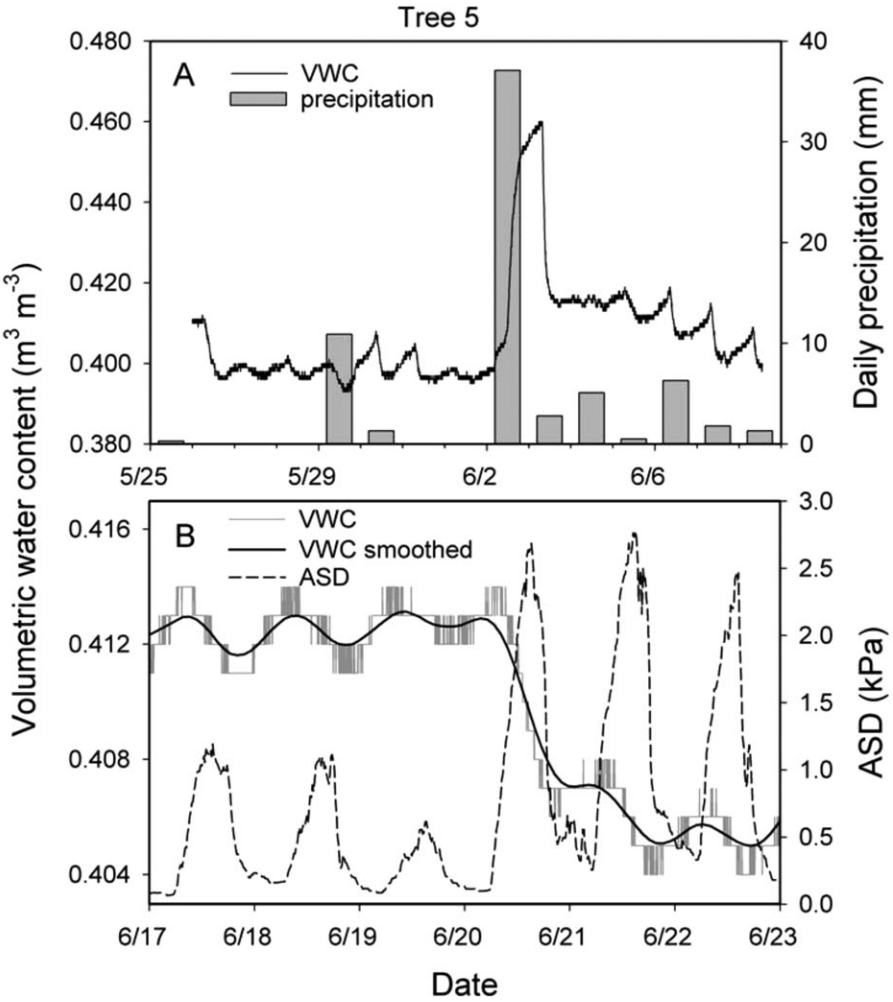

Fig. 2. (A) Recharge of trunk volumetric water content (VWC) in a representative tree during a significant rainfall event on 2 June 2012. (B) Substantial decrease in trunk VWC due to a heat wave occurred in the early summer (started on 20 June), during which air saturation deficit (ASD) of the atmosphere (Fisher Meteorological Station of Harvard Forest) was substantially higher than normal.

in small stem segments (1.7 MPa; $P > 0.05$, t-test). The dye perfusion result showed that air injection resulted in a large number of air filled non-conductive vessels in the xylem close to the injection point (Fig. 4B).

In trees that were still generating root pressure (prior to leaf flush), the post-air-injection VWC gradually increased before it decreased again due to leaf expansion (Fig. 5A and Supplementary Fig. S4A, B). Notably, after air injection, obvious diurnal fluctuations in VWC reoccurred (Fig. 5A and Supplementary Fig. S4A, B) in a pattern similar to those naturally occurring in March (Supplementary Fig. S3A), with higher values observed at daytime.

Air injection in early June, after leaves were fully expanded, also resulted in significant decrease in VWC but with a smaller magnitude relative to the injection before leaf flush (0.05 vs. 0.11 m^3 m^{-3}), which may resulted from an overall lower pre-injection VWC in June than in April. The large variation among trees in the pre-injection VWC (0.50–0.54 m^3 m^{-3} in the spring and 0.38–0.48 m^3 m^{-3} in the summer) may be also in part due to differences in sapwood depth and site soil water availability (the spring site located along a brook and the summer site located on a hill slope). These factors may have resulted in large differences in the absolute VWC change among trees during air injection. This problem may be partially solved by using shorter FDR probes in the future. For this reason the VWC change was expressed in a relative term, i.e. decrease in VWC under each pressure relative to the maximum VWC decrease under the highest applied pressure. In the freely transpiring trees, no significant recovery in VWC was observed (Fig. 5B), indicating a lack of xylem refilling in the absence of root pressure. In the stump of the tree that was cut down after air injection, VWC rapidly increased to a significantly higher level within an hour (Fig. 5B), reflecting the disappearance of xylem tension.

Xylem anatomy

Transverse sections showed that in the trunk xylem of *B. papyrifera* about 15% of the whole cross-sectional area is occupied by the lumens of diffusely distributed vessels and most other area is filled with fibres, with no more than 10% of the cross section occupied by thin lines of ray parenchyma (Supplementary Fig. S6). Average vessel diameter in trunk sapwood is much larger than in small branches (95.5 vs. 32.7 μm; $P < 0.001$).

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

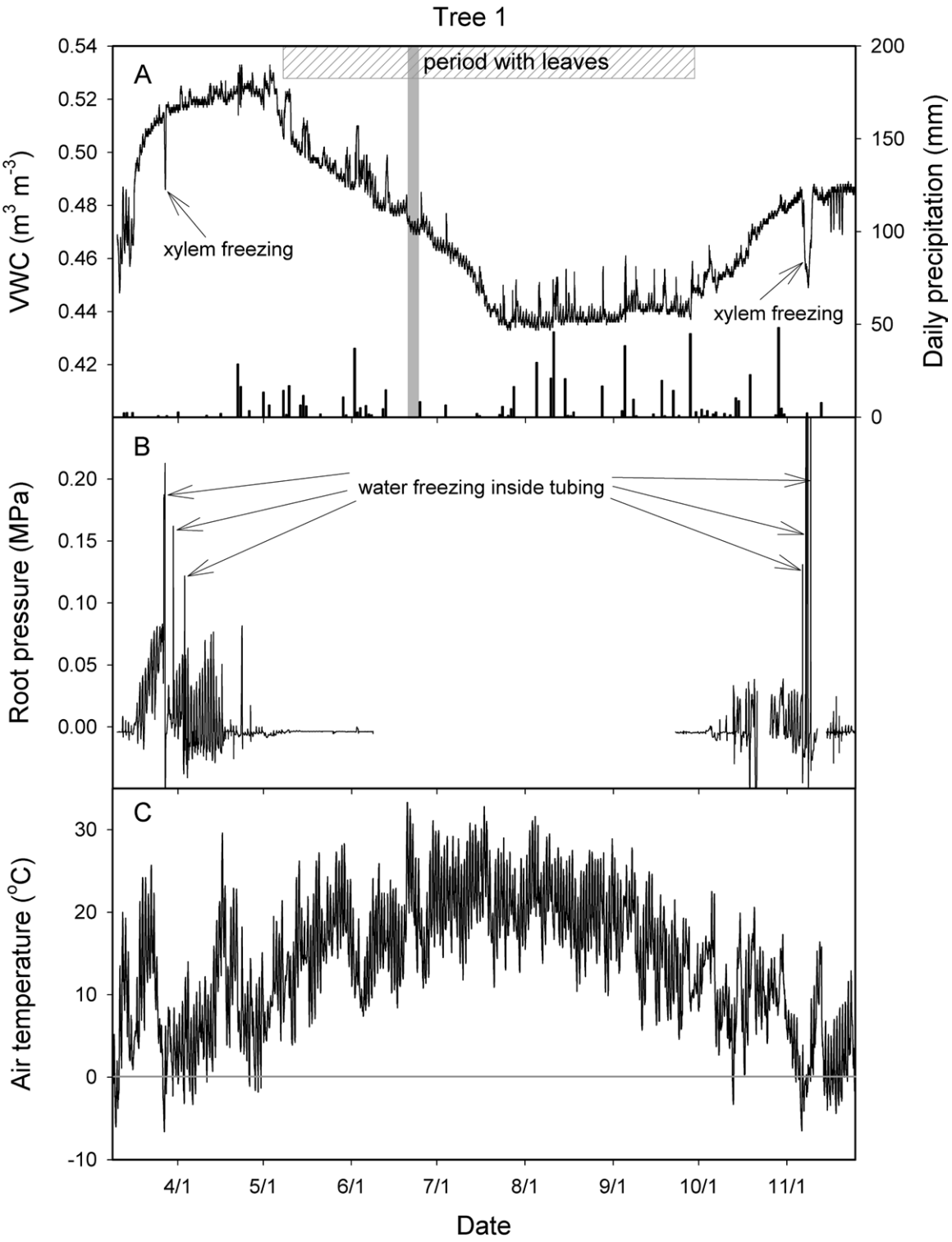

Fig. 3. Seasonal changes in a representative *B. papyrifera* tree of (A) trunk volumetric water content (VWC, thin line) and daily sums of precipitation (vertical black bars), (B) root pressure recorded in the spring and autumn of 2012, and (C) environmental air temperature. The vertical gray bar in (A) denotes the timing of the heat wave shown in Fig. 2B.The horizontal line in (C) marks air temperature of 0 °C.

Discussion

Embolism repair under root pressure in early spring

The trunk wood VWC data in this study showed that it took about 2 weeks from the first day of root pressure formation until the VWC reached stable maximum values (Fig. 1A), which is consistent with the timing of hydraulic conductivity re-establishment in other temperate tree species (Sperry *et al*., 1988; Miller-Rushing and Primack, 2008). The air injection and dye perfusion experiments also indicate that the VWC

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

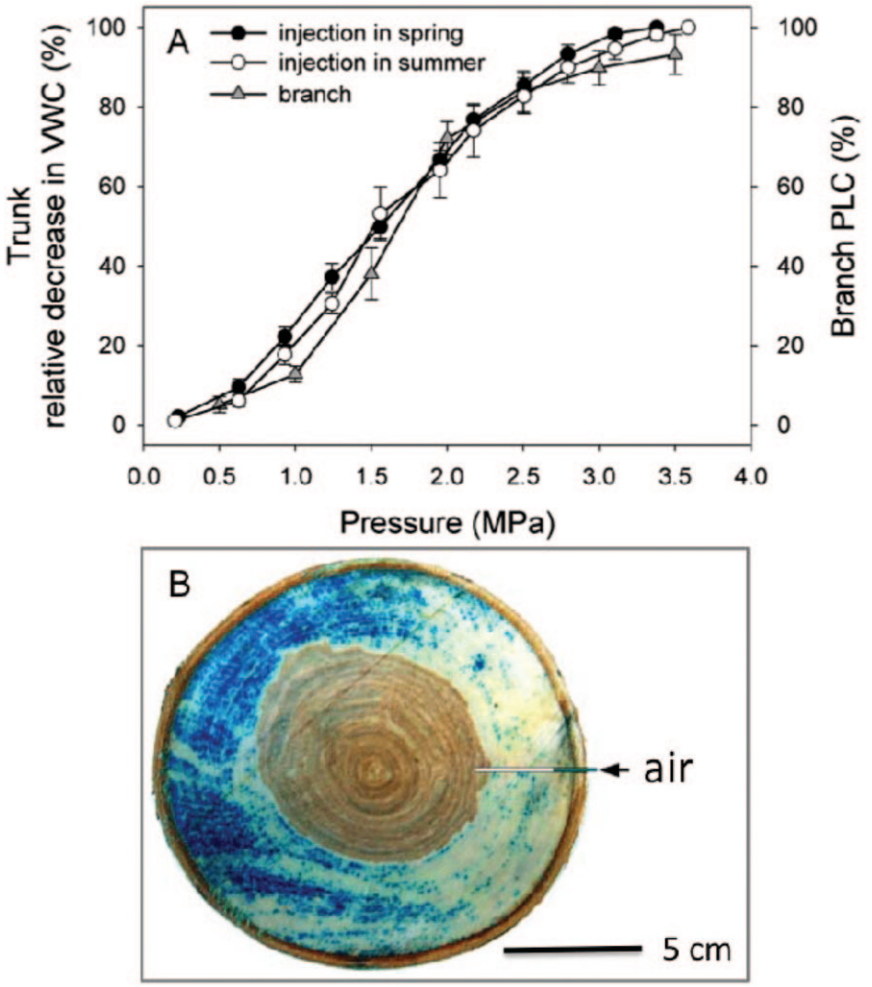

Fig. 4. (A) Percentage change in volumetric water content (VWC, relative to the total VWC decrease achieved at highest applied air pressure) as a function of air injection pressure measured on tree trunks and percentage loss of conductivity (PLC) as a function of centrifugal force measured on small branches. Error bars show ± 1 SE (n = 4 for trunk VWC in both spring and summer and n = 5 for branch PLC). (B) Dye perfusion showing that the xylem close to the injection site became cavitated heavily after air injection (this figure is available in colour at *JXB* online).

change in *B. papyrifera* trunks was related to xylem cavitation. The gradual increase of VWC after air injection in the early spring and a lack of increase in VWC after air injection when leaves were fully expanded further indicate that root pressure plays a critical role in the refilling of winter-embolized vessels in this species. Refilling of winter-embolized vessels requires significant positive pressure because these vessels are filled with CO_2- and O_2-enriched air (in cavitated vessels, water vapour is rapidly replaced by air), which is not easy to dissolve (Sperry *et al.*, 1987). It has been shown that in early spring when root pressure is prevented from reaching the crown, by overlapping saw cuts, hydraulic conductivity of *Betula* species cannot recover from winter levels (Sperry, 1993). A significant negative correlation between percentage loss of hydraulic conductivity and wood water content has been found in *Betula* species (Strati *et al.*, 2003), which is consistent with the winter embolism refilling in *B. papyrifera* trunks as measured by VWC increase using FDR sensors in the present study.

Unlike in vines, no evidence for gas expulsion through xylem 'leaks' has been found in trees (Sperry *et al.*, 1988; Hacke and Sauter, 1996). It seems that the dissolution of gas into xylem sap under positive pressure and the diffusion of gas to the outside surface of the branch are the major mechanisms for winter embolism repair in trees (Yang and Tyree, 1992). The results of this study showed that during the active refilling process in early spring, VWC showed large diurnal fluctuations (Supplementary Fig. S3A), which might be due to the shrinkage and expansion of air bubbles in the xylem in response to root pressure. The diurnal fluctuation in VWC diminished over a period of 2 weeks, which was likely due to the slow reduction in bubble size as root pressure forced gas into solution.

Trunk xylem vulnerability

The trunk air injection method is based on the same principles as the conventional air injection method (double-ended pressure sleeve) for determining hydraulic vulnerability in small branches, which measures decrease in hydraulic conductivity of hydrated stems or roots as a function of air

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

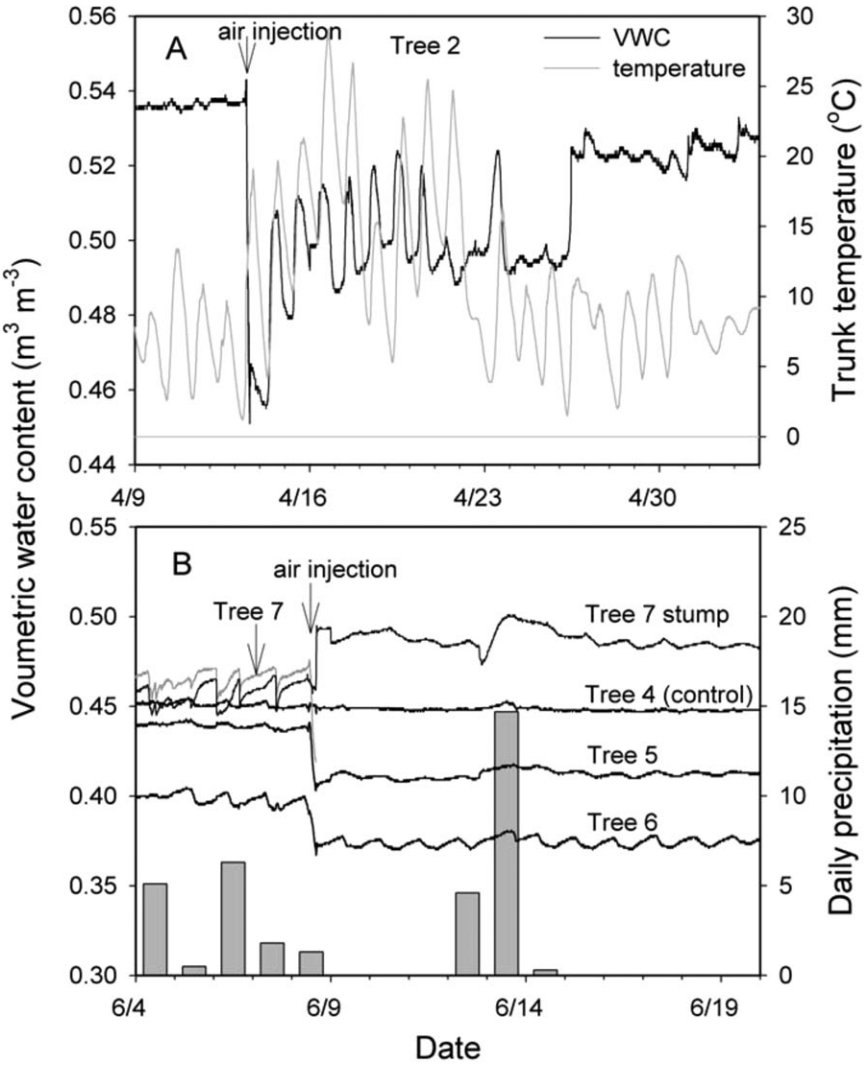

Fig. 5. (A) Decrease of trunk volumetric water content (VWC) due to air injection and its gradual recovery under root pressure in early spring in a representative tree. (B) VWC following air injection in early June in the absence of root pressure, showing no significant recovery of VWC in transpiring trees (thin lines, VWC; bars, daily sums of precipitation. Two sensors were installed in tree 7; one at *c.*70 cm above the ground (dark line shown as 'tree 7 stump') and another at breast height, which was removed when the tree was cut down after air injection (grey line shown as 'tree 7').

pressure surrounding the xylem (Sperry and Saliendra, 1994). The conventional air injection method estimates the pressure needed to induce cavitation by assuming that positive pressure will push air across the pit membranes when it is equal and opposite to the tension that would pull air across the pits in functioning xylem. This idea, which stems from the 'air seeding' hypothesis (Zimmermann, 1983), is strongly supported by the close correspondence between vulnerability curves obtained by air injection versus dehydration methods (Sperry *et al*., 1996). Similarly to branches measured with a double-ended pressure sleeve, the air injection of the trunk resulted in sigmoid curves when wood VWC was plotted against air pressure (Fig. 4A).

During the air injection treatment, the high-pressure gas propagates both radially and axially and the pressure builds up in the xylem around the injection point, which was larger than the volume of wood that the FDR moisture probe senses as evidenced by the dye perfusion results (Fig. 4B). The results suggest that the decrease of VWC in *B. papyrifera* during air injection was mainly due to water being forced out of the vessels other than the surrounding tissue, which is dominantly fibre matrix (Supplementary Fig. S6). Although the fibre lumens can contain a considerable amount of water, they may not be well connected to the vessels hydraulically, which otherwise would allow the stored water easily be pulled away by the transpiration stream undermining their water storage function (Holbrook, 1995). Under a pressure higher than that needed for 'air-seeding', water contained in the affected vessels can easily be pushed axially to the neighbouring vessels up or down stream of the injection point through pits, while the much shorter length of the fibre cells relative to the vessels may have resulted in the minor contribution of water loss in fibre cells during injection. If the discharge of fibre capillary storage had contributed to the trunk VWC decrease during

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

air injection, a large VWC drop would have been observed at a low pressure range, 0 –0.6 MPa, which is the theoretical functioning range for fibre water storage (Tyree and Yang, 1990). Together with the dye perfusion results and the agreement between the observed maximum VWC drop during air injection (on average 0.11 $m^3 m^{-3}$ for the March injection) and the calculated volume fraction of vessel lumen inside the sapwood (15%), it is reasonable to conclude that the measured VWC change during air injection was mainly due to embolism formation in the xylem. The resulting curves from this method can therefore be used as a surrogate for trunk xylem hydraulic 'vulnerability curves'.

Correspondence in P_{50} (the pressure at which there is a 50% loss of conductance or reduction in relative VWC change) between small branch hydraulic vulnerability curves and the trunk air injection curves suggests that trunk and branch xylem are equally vulnerable to drought-induced cavitation (Fig. 4A), although the vessel diameter in trunks is much greater than that of small branches. This suggests, at least for *B. papyrifera*, the absence of vulnerability segmentation between the tree trunk and terminal branches. Contradicting results have been found in different species in comparing hydraulic vulnerability of branches of different diameters (Cochard, 1992; Tyree *et al*., 1993; Sperry and Saliendra, 1994; Sperry and Ikeda, 1997; Choat *et al*., 2005), which indicates that vulnerability segmentation is not a general phenomenon. The ambiguous relationship between conduit size and vulnerability to drought-induced cavitation is consistent with the lack of a necessary causal link between them. Rather, cavitation appears to be structurally correlated with inter-conduit pit membranes that determine the critical pressure or tension for air seedling (Sperry *et al*., 1996).

The lack of apparent vulnerability segmentation, however, does not necessarily imply that the trunk and peripheral xylem are equally susceptible to drought-induced cavitation in these trees. It has been found that a larger fraction of whole-xylem hydraulic resistance resides in the branches than in the trunk (Edwards *et al*., 1986; Tyree, 1988; Tyree and Sperry, 1988; Tyree *et al*., 1991; Yang and Tyree, 1994), which results in much steeper water potential gradients in the small terminal branches than in big stems or the trunk of a transpiring tree. Higher water potential in big stems or the trunk itself is likely to confer a lower risk of cavitation compared to that in small terminal branches. It has also been found that leaves are more vulnerable to drought-induced hydraulic failure than stems (Hao *et al*., 2008), which suggest that vulnerability segmentation may mainly occur between terminal branches and leaves rather than between stems of different sizes (Choat *et al*., 2005).

Xylem water storage and plant water balance

It has long been known that trees can store water in their xylem tissues with a seasonal and diurnal rhythm of storage and depletion (Landsberg *et al*., 1976; Roberts, 1976; Waring *et al*., 1979). Although sap flow and stem dendrometer techniques have been used to estimate the contribution of trunk water storage to daily transpiration (e.g. Goldstein *et al*., 1998; Scholz *et al*., 2008), no previous study has provided such a high temporal resolution and continuous measurements nondestructively in functioning trees over a relatively long period. By using the FDR moisture sensor, critical methodological problems inherent in measuring diurnal and seasonal changes of wood water content by sampling with increment coring equipment were avoided (Waring and Running, 1978; Waring *et al*., 1979). Whenever wood fibres and conduits are cut open, the water contained in them is held only by the capillary forces generated by the radius of curvature of the air-water interfaces of the severed cells and thus can be pulled away by tension in the neighbouring functional xylem. The FDR probe installation used in the present study does only minor damage to the xylem, relative to a much larger volume of untouched wood tissues that the probe senses (volume of influence is 0.3 l), and thus provides a more accurate measurement of diurnal and seasonal changes of xylem tissue water content.

The diurnal fluctuations in trunk VWC indicate the importance of internal water storage to daily water balance in *B. papyrifera* trees. Withdrawal of water from internal storage compartments can account for 10–50% daily water use in trees depending on species, ecosystem type, and tree size (Goldstein *et al*., 1998; Meinzer *et al*., 2004; Scholz *et al*., 2007, 2011). The use of internal stored water close to the site of transpiration reduces the apparent hydraulic resistance along the plant water transport pathway and can therefore buffer temporal changes in leaf water status, which in turn can reduce the extent of stomata limitation to photosynthesis (Meinzer, 2002). Water storage as a homeostatic mechanism may be especially important in tall trees as hydraulic resistance increases with tree height (Goldstein *et al*., 1998; Phillips *et al*., 2003). Furthermore, daily water withdrawal from storage at times of peak transpiration with later recharge allows roots to take up water at intermediate rates over a longer period and thus plants can meet their water needs with less roots than would otherwise be necessary (Tyree and Yang, 1990).

The VWC changed over longer time periods, such as recharge after significant rainfalls and gradual decline thereafter and the dramatic decrease that occurred during a summer heat wave (Fig. 2), which indicates the importance of water storage to plant water balance in longer terms. More interestingly, the water content of the trunks had an overall gradual decrease from leaf flush through the end of the growing season, which may largely be due to changes in fibre capillary water storage since parenchyma ray cells only occupy a very small portion of the xylem cross-section in *B. papyrifera*. The intercellular spaces inside fibres, fibre tracheids, or tracheids can provide considerable water storage capacitance in trees in the form of capillary storage (Zimmermann, 1983; Tyree and Yang, 1990). Partially air-filled fibres in the xylem of hardwood trees are ideal for water storage, because their slender tips can hold a considerable amount of water. Because capillary tension is inversely related to diameter, the volume of water trapped inside fibres is negatively related to xylem tension; as the tension increases, the menisci of water bubbles inside the fibre lumens are pulled toward the narrower tips

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

where larger capillary forces re-establish balance with tension. When water is available and xylem tension decreases, the capillary tension in the fibre causes air bubbles to shrink and more water can be stored in the fibres.

Water storage by capillarity only provides an appreciable amount of water over a relatively narrow range of water potentials, between 0 and –0.6 MPa (Tyree and Yang, 1990). Water potential measured on *Betula occidentalis* grown in Utah using stem psychrometers showed that midday trunk water potential in August was about –0.6 MPa (Sperry and Pockman, 1993). In the more humid environment of the current study site, one can safely predict that the *B. papyrifera* trunk water potential rarely departs from the range of 0 to –0.6 MPa, which is not low enough to induce significant wood water loss due to vessel cavitation according the vulnerability curves (Fig. 4A) but matches the water potential range in which capillary water storage functions. The slower VWC increase under root pressure in the autumn relative to that happened in the spring also suggest that the seasonal water content decrease in the trunk xylem was not due to cavitation but likely due to the depletion of stored water in fibres, which might be more difficult to refill.

In conclusion, FDR moisture sensors have the potential to detect important processes related to the short- and long-term dynamics of plant water balance in tree trunks. The trunk air injection technique developed based on the FDR measurement of water content seems to be a reliable technique for estimating xylem vulnerability in tree trunks or large stems. Trees subjected to air injection in the early spring in the presence of root pressure partly recovered VWC implying vessel refilling, while those trees air injected in the absence of root pressure showed no recovery of VWC. The contrasting post-air-injection responses of xylem VWC in trees with and without root pressure indicate the essential role of root pressure in embolism refilling. Comparison of the vulnerability curves derived from the trunk air injection and conventional branch hydraulic vulnerability curves found no evidence of vulnerability segmentation between the trunk and small branches in *B. papyrifera*.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. The apparatus used for the trunk air injection experiment.

Supplementary Fig. S2. Calibration of the FDR sensor.

Supplementary Fig. S3. Patterns of diurnal change in trunk volumetric water content measured at different times during the year.

Supplementary Fig. S4. Seasonal change of volumetric water content in trunk xylem of trees 2 and 3 and daily sums of precipitation.

Supplementary Fig. S5. Seasonal change of volumetric water content (VWC) in trunk xylem of trees 4, 5, and 6 and daily sums of precipitation.

Supplementary Fig. S6. A transverse section of *B. papyrifera* trunk xylem.

Acknowledgements

This work was supported by grants from the David Rockefeller Center for Latin American Studies (GG) and the Sustainability Science Program (GYH) from Harvard University. GYH is currently supported by the Katharine H. Putnam Fellowship at the Arnold Arboretum of Harvard University. Harvard Forest is gratefully acknowledged for supporting this study. The authors thank the two anonymous reviewers for their constructive comments.

References

Alder NN, Pockman WT, Sperry JS, Nuismer S. 1997. Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* **48,** 665–674.

Améglio T, Bodet C, Lacointe A, Cochard H. 2002. Winter embolism, mechanisms of xylem hydraulic conductivity recovery and springtime growth patterns in walnut and peach trees. *Tree Physiology* **22,** 1211–1220.

Choat B, Lahr EC, Melcher PJ, Zwieniecki MA, Holbrook NM. 2005. The spatial pattern of air seeding thresholds in mature sugar maple trees. *Plant, Cell and Environment* **28,** 1082–1089.

Cobb AR, Choat B, Holbrook NM. 2007. Dynamics of freeze-thaw embolism in *Smilax rotundifolia* (Smilacaceae). *American Journal of Botany* **94,** 640–649.

Cochard H. 1992. Vulnerability of several conifers to air embolism. *Tree Physiology* **11,** 73–83.

Cochard H, Tyree MT. 1990. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiology* **6,** 393–407.

Czarnomski NM, Moore GW, Pypker TG, Licata J, Bond BJ. 2005. Precision and accuracy of three alternative instruments for measuring soil water content in two forest soils of the Pacific Northwest. *Canadian Journal of Forest Research* **35,** 1867–1876.

Edwards WRN, Jarvis PG, Landsberg JJ, Talbot H. 1986. A dynamic model for studying flow of water in single trees. *Tree Physiology* **1,** 309–324.

Ewers FW. 1985. Xylem structure and water conduction in conifer trees, dicot trees, and lianas. *IAWA Bulletin* **6,** 309–317.

Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A. 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell and Environment* **21,** 397–406.

Hacke U, Sauter JJ. 1996. Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees. *Oecologia* **105,** 435–439.

Hammel HT. 1967. Freezing of xylem sap without cavitation. *Plant Physiology* **42,** 55–66.

Hao G-Y, Hoffmann WA, Scholz FG, Bucci SJ, Meinzer FC, Franco AC, Cao K-F, Goldstein G. 2008. Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* **155,** 405–415.

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

Holbrook NM. 1995. Stem water storage. In: BL Gartner, ed, *Plant stems: physiology and functional morphology* . San Diego: Academic Press, pp 151–174.

James SA, Meinzer FC, Goldstein G, Woodruff D, Jones T, Restom T, Mejia M, Clearwater M, Campanello P. 2003. Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia* **134,** 37–45.

Jaquish LL, Ewers FW. 2001. Seasonal conductivity and embolism in the roots and stems of two clonal ring-porous trees, *Sassafras albidum* (Lauraceae) and *Rhus typhina* (Anacardiaceae). *American Journal of Botany* **88,** 206–212.

Kizito F, Campbell CS, Campbell GS, Cobos DR, Teare BL, Carter B, Hopmans JW. 2008. Frequency, electrical conductivity and temperature analysis of a low-cost capacitance soil moisture sensor. *Journal of Hydrology* **352,** 367–378.

Landsberg JJ, Blanchard TW, Warrit B. 1976. Studies on movement of water through apple trees. *Journal of Experimental Botany* **27,** 579–596.

Magnani F, Borghetti M. 1995. Interpretation of seasonal changes of xylem embolism and plant hydraulic resistance in *Fagus sylvatica*. *Plant, Cell and Environment* **18,** 689–696.

Meinzer FC. 2002. Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell and Environment* **25,** 265–274.

Meinzer FC, James SA, Goldstein G. 2004. Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. *Tree Physiology* **24,** 901–909.

Miller-Rushing AJ, Primack RB. 2008. Effects of winter temperatures on two birch (*Betula*) species. *Tree Physiology* **28,** 659–664.

Oertli JJ. 1971. The stability of water under tension in the xylem. *Zeitschrift für Pflanzenphysiologie* **65,** 195–209.

Phillips NG, Ryan MG, Bond BJ, McDowell NG, Hinckley TM, Cermák J. 2003. Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* **23,** 237–245.

Roberts J. 1976. Examination of the quantity of water stored in mature *Pinus sylvestris* L. trees. *Journal of Experimental Botany* **27,** 473–479.

Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2007. Biophysical properties and functional significance of stem water storage tissue in neo-tropical savanna trees. *Plant, Cell and Environment* **30,** 236–248.

Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F. 2008. Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. *Tree Physiology* **28,** 469–480.

Scholz FG, Phillips NG, Bucci SJ, Meinzer FG, Goldstein G. 2011. Hydraulic capacitance: biophysics and functional significance of internal water sources in relation to tree size. In: FC Meinzer, B Lachenbruch, TE Dawson, eds, *Size- and age-related changes in tree structure and function* . New York: Springer, pp 341–362.

Sperry JS. 1993. Winter xylem embolism and spring recovery in *Betula cordifolia, Fagus grandifolia, Abies balsamifera,* and *Picea rubens*. In: A Raschi, M Borghetti, J Grace, eds, *Water transport in plants under climatic stress* . Cambridge: Cambridge University Press, pp 86–98.

Sperry JS, Donnelly JR, Tyree MT. 1988. Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). *American Journal of Botany* **75,** 1212–1218.

Sperry JS, Holbrook NM, Zimmermann MH, Tyree MT. 1987. Spring filling of xylem vessels in wild grapevine. *Plant Physiology* **83,** 414–417.

Sperry JS, Ikeda T. 1997. Xylem cavitation in roots and stems of Douglas-fir and white fir. *Tree Physiology* **17,** 275–280.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* **75,** 1736–1752.

Sperry JS, Pockman WT. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16,** 279–287.

Sperry JS, Saliendra NZ. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **17,** 1233–1241.

Sperry JS, Saliendra NZ, Pockman WT, Cochard H, Cruiziat P, Davis SD, Ewers FW, Tyree MT. 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant, Cell and Environment* **19,** 427–436.

Sperry JS, Sullivan JEM. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiology* **100,** 605–613.

Strati S, Patiño S, Slidders C, Cundall EP, Mencuccini M. 2003. Development and recovery from winter embolism in silver birch: seasonal patterns and relationships with the phenological cycle in oceanic Scotland. *Tree Physiology* **23,** 663–673.

Tyree MT. 1988. A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. *Tree Physiology* **4,** 195–217.

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Améglio T. 1993. Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell and Environment* **16,** 879–882.

Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119,** 345–360.

Tyree MT, Snyderman DA, Wilmot TR, Machado J-L. 1991. Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*). Data, models, and a comparison with two temperate species (*Acer saccharum* and *Thuja occidentalis*). *Plant Physiology* **96,** 1105–1113.

Tyree MT, Sperry JS. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiology* **88,** 574–580.

Tyree MT, Yang SD. 1990. Water-storage capacity of *Thuja*, *Tsuga* and *Acer* stems measured by dehydration isotherms. The contribution of capillary water and cavitation. *Planta* **182,** 420–426.

Vogel S. 1988. *Life's devices: the physical world of animals and plants* . Princeton: Princeton University Press.

Wang J, Ives NE, Lechowicz MJ. 1992. The relation of foliar phenology to xylem embolism in trees. *Functional Ecology* **6,** 469–475.

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016

Waring RH, Running SW. 1978. Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. *Plant, Cell and Environment* **1,** 131–140.

Waring RH, Whitehead D, Jarvis PG. 1979. The contribution of stored water to transpiration in Scots pine. *Plant, Cell and Environment* **2,** 309–317.

Yang S, Tyree MT. 1992. A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant, Cell and Environment* **15,** 633–643.

Yang SD, Tyree MT. 1994. Hydraulic architecture of *Acer saccharum* and *A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. *Journal of Experimental Botany* **45,** 179–186.

Zimmermann MH. 1978. Hydraulic architecture of some diffuse-porous trees. *Canadian Journal of Botany-Revue Canadienne De Botanique* **56,** 2286–2295.

Zimmermann MH. 1983. *Xylem structure and the ascent of sap* . Berlin, Heidelberg, New York, Tokyo: Springer-Verlag.

Downloaded from http://jxb.oxfordjournals.org/ at Ernst Mayr Library of the Museum Comp Zoology, Harvard University on May 12, 2016