Letters

Recent advances in tree hydraulics highlight the ecological significance of the hydraulic safety margin

Introduction

Drought-induced cavitation resistance varies considerably between tree species and forest ecosystems (Maherali *et al.*, 2004; Delzon *et al.*, 2010) and is closely linked to survival under severe drought in both conifers (Brodribb & Cochard, 2009; Brodribb *et al.*, 2010) and angiosperms (Kursar *et al.*, 2009; Anderegg *et al.*, 2012; Barigah *et al.*, 2013; Urli *et al.*, 2013). Choat *et al.* (2012) recently reported that most trees operate very close to their threshold of cavitation, leaving them potentially vulnerable to drought-induced mortality in a warmer/drier world (Engelbrecht, 2012). Indeed, species growing in dry environments are more resistant to drought-induced cavitation (more negative water potential at 50% cavitation, P_{50}) but experience a more negative minimum water potential (P_{min}) than those growing in wet environments. The so-called hydraulic safety margin, the difference between the level of water stress experienced by a species in the field (P_{min}) and the level of water stress leading to hydraulic failure, is, therefore, remarkably narrow, whatever the forest species and biome considered (Choat *et al.*, 2012). This pattern provides clues to the global drought-induced mortality currently observed, even in very wet environments, such as tropical forests (Allen *et al.*, 2010).

Klein *et al.* (2014) play down the functional significance of the hydraulic safety margin in the vulnerability of forests to drought, pointing out the important role played by additional mechanisms, such as the ability of trees to repair embolism. While it is obvious that drought-induced forest dieback is a complex process involving a number of biotic and abiotic factors, we would like to draw the attention of scientists to the state of evidence for embolism repair, thereby guiding research on tree drought resistance into the most relevant and fruitful directions.

A misunderstanding concerning xylem embolism

Recent advances in tree hydraulics have demonstrated that, contrary to what was previously believed, embolism and repair may be far from routine in trees (Cochard *et al.*, 2013; Wheeler *et al.*, 2013). Indeed, several studies had previously concluded that trees seemed to be highly vulnerable to cavitation (Salleo *et al.*, 1996), with P_{min} values much more negative than P_{50}, leading to a high level of xylem cavitation and an apparently negative safety margin, but with recovery on a daily basis. Methodological problems that have only recently been discovered put this alternative conception of tree functioning under question.

Daily cavitation is not routine in trees

Daily patterns of embolism formation and apparent recovery in well watered trees (Canny, 1997; Zwieniecki & Holbrook, 1998; Zufferey *et al.*, 2011) are in some cases known to be biased by sampling procedures. Canny (1997) used cryo-scanning electron microscopy (SEM) to provide evidence of the occurrence of considerable daily variation in cavitation, but Cochard *et al.* (2000) refuted this conclusion by demonstrating that cavitation occurred in stretched sap upon freezing of the sample. Similarly, Zwieniecki & Holbrook (1998) detected large daily fluctuations in xylem hydraulic conductance that were presumably caused by cavitation and suggested that embolism removal occurred in two tree species (*Fraxinus americana* and *Acer rubrum*) despite the existence of tension within the xylem. Wheeler *et al.* (2013) have also recently demonstrated in the same two species that the formation of embolism following the cutting of branches under water is overestimated when xylem tensions are large. These authors concluded that the idea that many plants are prone to embolism under typical midday water potentials should be reconsidered. Without further work that explicitly avoids such artifacts, we have no unequivocal evidence for daily cavitation (Rockwell *et al.*, 2014). Independent observations in intact seedlings by use of high-resolution computed tomography are urgently needed to unravel this issue in trees as it has been done in grapevine (McElrone *et al.*, 2012; Brodersen *et al.*, 2013).

Issues concerning highly vulnerable species

Cochard *et al.* (2013) conducted an extensive literature survey on the methods used to estimate cavitation resistance and concluded that the long-vessel species reported to be highly vulnerable to cavitation had actually been evaluated with biased techniques. New methods have been introduced based on air injection (Cochard *et al.*, 1992) or centrifugation (Alder *et al.*, 1997; Cochard, 2002) for the assessment of cavitation resistance, and these attractive methodologies are now widely used. The *in situ* flow centrifuge technique (Cavitron) has been shown to overestimate vulnerability to cavitation in species with very long vessels (Cochard *et al.*, 2010; Martin-St Paul *et al.*, 2014; Wang *et al.*, 2014) due to open vessel artifact. We concluded that this method as well as the air injection technique (Ennajeh *et al.*, 2011) are not appropriate for use in the measurement of cavitation resistance in species with long vessels. By contrast, other studies assessing the validity of the static centrifuge technique (in which flow measurements are made outside the

centrifuge) in large vessels of ring-porous trees (Christman *et al.*, 2012; Sperry *et al.*, 2012; Tobin *et al.*, 2013) did not find support for the open vessel artifact hypothesis. However, a recent study has shown that all centrifuge techniques, independent of the rotor design, are prone to the open vessel artifact (Torres-Ruiz *et al.*, 2014). Direct observations of vessel function using high-resolution computed tomography have also confirmed the remarkable ability of long vessels to resist cavitation (Torres-Ruiz *et al.*, 2014). We therefore recommend interpretation of vulnerability curves showing rapid decline in conductance at high pressure (between 0 and −1 MPa) with caution, before a clear understanding of the open vessel artifact can be obtained.

Problematic evidence of xylem refilling under tension

In the 1990s, rapid xylem refilling in stems has been reported under negative pressure in a few species (Salleo *et al.*, 1996, 2004; Holbrook & Zwieniecki, 1999; Tyree *et al.*, 1999). Direct observations of embolism repair under apparently negative pressures have also recently been reported in grapevine (Brodersen *et al.*, 2010). However, as appropriately pointed out by Sperry (2013), xylem pressure was not precisely measured in the stem in which refilling was observed, but on stressed distal leaves that may have not been hydraulically connected or equilibrated with the vascular system. We still need direct measurements of xylem pressure at the site of refilling based on more appropriate techniques, such as the use of a stem psychrometer or xylem pressure probe. All together, these recent results highlighting methodological artifacts call for much greater care in assessing the presence of refilling capable of reversing drought-induced embolism in trees, and its prevalence may have been greatly overestimated (Cochard & Delzon, 2013).

These findings suggest that cavitation may occur only when xylem pressure falls below a threshold pressure that trees experience only after prolonged episodes of drought and that refilling under tension cannot keep a plant from runaway cavitation. Drought-induced xylem cavitation is thus a symptom of distress. Because the prevalence of the low-cavitation resistance and high-repair capacity paradigm could result from erroneous observations, we propose the high-cavitation-resistance paradigm as the default framework for understanding tree hydraulics and water relations (Fig. 1). Accordingly, tree survival would be unlikely to be dependent on xylem refilling when xylem water potential drops beyond the hydraulic safety margin, as suggested by Klein *et al.* (2014).

The functional and ecological significance of the hydraulic safety margin

Hydraulic safety margins can be defined in different ways (see Meinzer *et al.*, 2009). From an ecological perspective, it is relevant to define a metric relative to a threshold for tree mortality (see McDowell, 2011, for a review). In conifers, the lethal level of cavitation was found to be close to 50% of embolized conduits in seedling stems (Brodribb & Cochard, 2009; Brodribb *et al.*, 2010), the P_{50} safety margin ($P_{min} - P_{50}$) being the most relevant metric. In a pine–juniper woodland, Plaut *et al.* (2012) recently demonstrated in adult trees that the species less prone to drought-induced
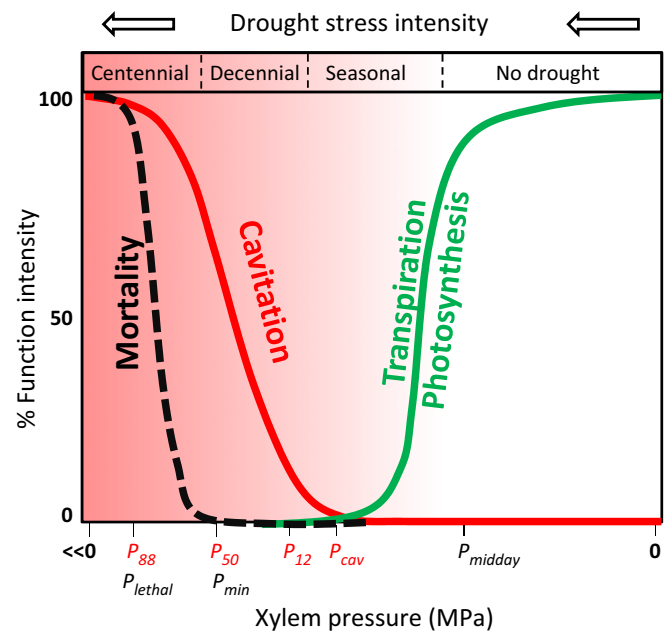

Fig. 1 Functional significance of xylem cavitation in angiosperm trees. Drought stress-induced cavitation in trees is a physical process that occurs when the xylem pressure drops below a threshold value (P_{cav}). Under well watered conditions, xylem pressure (P_{midday}) is much higher than P_{cav}, and stomata are wide open, leading to high rates of transpiration and photosynthesis. When water stress increases under summer drought conditions, the stomata close, thereby maintaining xylem pressure slightly below P_{cav} (= P_{12}). Drought may be more intense, resulting in a spread of cavitation into the xylem conduits, but trees can recover by producing new conduits. Exceptionally, drought may be of extreme intensity, leading to total hydraulic failure and tree death. This happens when xylem pressure reaches the lethal water potential (P_{lethal}) (close to P_{88} and P_{50} for angiosperms and conifers, respectively). The minimum water potential experienced by a species in the field (P_{min}) is, on average, 2 MPa higher than P_{88} for angiosperms (2 MPa higher than P_{50} for conifers, see Choat *et al.*, 2012).

mortality (*Juniperus monosperma*) exhibited a greater safety margin at the whole pant level compared to the vulnerable *Pinus edulis*. Data for angiosperms have recently become available (Anderegg *et al.*, 2012; Barigah *et al.*, 2013; Urli *et al.*, 2013), and have shown that the point of no return is closer to 88% cavitation in the stem. A more conservative lethal safety margin, calculated as ($P_{min} - P_{88}$), may be more appropriate for angiosperms (Choat, 2013). Even though more work is needed to characterize hydraulic failure threshold in different trees species, we suggest that there is a remarkable convergence in lethal hydraulic safety margin across all species and all biomes (combine P_{50} safety margin in Fig. 2a for conifers and P_{88} safety margin in Fig. 2b for angiosperms in Choat *et al.*, 2012). This hydraulic safety margin for mortality is overall positive (+2 MPa), suggesting that the point of no return is crossed only after an exceptionally intense and deadly episode of drought. Trees can recover partially or totally from the deleterious effects of water stress until they reach this lethal threshold. However the mechanism underlying this recovery is not necessarily xylem refilling, and is more likely to involve an ability to form new functional xylem conduits (Brodribb *et al.*, 2010).

Use of the species minimum water potential (instead of the water potential associated with stomatal closure) makes the estimate of

hydraulic safety margin robust across ecosystems and biomes. Indeed, P_{min} is altered by soil, but also by atmospheric water stress, taking the variability of both soil types and vapor pressure deficit across biomes into account. Thus, although not all forest biomes experience similar atmospheric and soil droughts, these differences are fully integrated into the hydraulic safety margin through the use of P_{min}. However, we now need to quantify the variation of safety margin within species, as the populations growing at species' warm/dry range margins would be expected to have a narrower safety margin. We hypothesize that these populations experience a more negative P_{min} (drier conditions), whereas it has been reported that resistance to cavitation does not vary between populations across the entire range of a species distribution (Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2011, 2013).

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References

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

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg E. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.

Anderegg WR, Berry JA, Smith DD, Sperry JS, Anderegg LD, Field CB. 2012. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences, USA* 109: 233–237.

Barigah TS, Charrier O, Douris M, Bonhomme M, Herbette S, Améglio T, Fichot R, Brignolas F, Cochard H. 2013. Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. *Annals of Botany* 112: 1431–1437.

Brodersen CR, McElrone AJ, Choat B, Lee EF, Shackel KA, Matthews MA. 2013. *In vivo* visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant Physiology* 161: 1820–1829.

Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA. 2010. The dynamics of embolism repair in xylem: *in vivo* visualizations using high-resolution computed tomography. *Plant Physiology* 154: 1088–1095.

Brodribb TJ, Bowman D, Nichols S, Delzon S, Burlett R. 2010. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytologist* 188: 533–542.

Brodribb TJ, Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* 149: 575–584.

Canny M. 1997. Vessel contents during transpiration: embolisms and refilling. *American Journal of Botany* 84: 1223–1230.

Choat B. 2013. Predicting thresholds of drought-induced mortality in woody plant species. *Tree Physiology* 33: 669–671.

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG et al. 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491: 752–755.

Christman MA, Sperry JS, Smith DD. 2012. Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. *New Phytologist* 193: 713–720.

Cochard H. 2002. A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant, Cell & Environment* 25: 815–819.

Cochard H, Badel E, Herbette S, Delzon S, Choat B, Jansen S. 2013. Methods for measuring plant vulnerability to cavitation: a critical review. *Journal of Experimental Botany* 64: 4779–4791.

Cochard H, Bodet C, Améglio T, Cruiziat P. 2000. Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or artifacts? *Plant Physiology* 124: 1191–1202.

Cochard H, Cruiziat P, Tyree MT. 1992. Use of positive pressures to establish vulnerability curves. Further support for the air-seeding hypothesis and implications for pressure-volume analysis. *Plant Physiology* 100: 205–209.

Cochard H, Delzon S. 2013. Hydraulic failure and repair are not routine in trees. *Annals of Forest Science* 70: 659–661.

Cochard H, Herbette S, Barigah T, Badel E, Ennajeh M, Vilagrosa A. 2010. Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. *Plant, Cell & Environment* 33: 1543–1552.

Delzon S, Douthe C, Sala A, Cochard H. 2010. Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. *Plant, Cell & Environment* 33: 2101–2111.

Engelbrecht BM. 2012. Plant ecology: forests on the brink. *Nature* 491: 675–677.

Ennajeh M, Simões F, Khemira H, Cochard H. 2011. How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? *Physiologia Plantarum* 142: 205–210.

Holbrook NM, Zwieniecki MA. 1999. Embolism repair and xylem tension: do we need a miracle? *Plant Physiology* 120: 7–10.

Klein T, Yakir D, Buchmann N, Grünzweig JM. 2014. Towards an advanced assessment of the hydrological vulnerability of forests to climate change-induced drought. *New Phytologist* 201: 712–716.

Kursar TA, Engelbrecht BM, Burke A, Tyree MT, Omari EI, B, Giraldo JP. 2009. Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Functional Ecology* 23: 93–102.

Lamy J-B, Bouffier L, Burlett R, Plomion C, Cochard H, Delzon S. 2011. Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range. *PLoS ONE* 6: e23476.

Lamy JB, Delzon S, Bouche PS, Alia R, Vendramin GG, Cochard H, Plomion C. 2013. Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. *New Phytologist* 201: 874–886.

Maherali H, Pockman WT, Jackson RB. 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85: 2184–2199.

Martínez-Vilalta J, Cochard H, Mencuccini M, Sterck F, Herrero A, Korhonen J, Llorens P, Nikinmaa E, Nolè A, Poyatos R. 2009. Hydraulic adjustment of Scots pine across Europe. *New Phytologist* 184: 353–364.

Martin-St Paul NK, Longepierre D, Huc R, Delzon S, Burlett R, Joffre R, Rambal S, Cochard H. 2014. How reliable are methods to assess xylem vulnerability to cavitation? The issue of "open vessel" artifact in oak. *Tree Physiology*, in press.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155: 1051–1059.

McElrone A, Brodersen C, Alsina M, Drayton W, Matthews M, Shackel K, Wada H, Zufferey V, Choat B. 2012. Centrifuge technique consistently overestimates vulnerability to water stress-induced cavitation in grapevines as confirmed with high-resolution computed tomography. *New Phytologist* 196: 661–665.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* 23: 922–930.

Plaut JA, Yepez EA, Hill J, Pangle R, Sperry JS, Pockman WT, McDowell NG. 2012. Hydraulic limits preceding mortality in a piñon–juniper woodland under experimental drought. *Plant, Cell & Environment* 35: 1601–1617.

Rockwell FE, Wheeler JK, Holbrook NM. 2014. Cavitation and its discontents: opportunities for resolving current controversies. *Plant Physiology*. doi: http://dx.doi.org/10.1104/pp.113.233817.

Salleo S, Lo Gullo M, Paoli D, Zippo M. 1996. Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytologist* 132: 47–56.

Salleo S, Lo Gullo M, Trifilo P, Nardini A. 2004. New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. *Plant, Cell & Environment* 27: 1065–1076.

Sperry J. 2013. Cutting-edge research or cutting-edge artifact? An overdue control experiment complicates the xylem refilling story. *Plant, Cell & Environment* 36: 1916–1918.

Sperry JS, Christman MA, Torres-Ruiz JM, Taneda H, Smith DD. 2012. Vulnerability curves by centrifugation: is there an open open vessel artefact, and are 'r' shaped curves necessarily invalid? *Plant, Cell & Environment* **35**: 601–610.

Tobin MF, Pratt RB, Jacobsen AL, De Guzman ME. 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. *Plant Biology (Stuttgart, Germany)* **15**: 496–504.

Torres-Ruiz JM, Cochard H, Mayr S, Beikircher B, Diaz-Espejo A, Rodriguez-Dominguez CM, Badel E, Fernández JE. 2014. Vulnerability to cavitation in *Olea europaea* current-year shoots: further evidence of an open-vessel artefact associated with centrifuge and air-injection techniques. *Physiologia Plantarum*. doi: 10.1111/ppl.12185.

Tyree MT, Salleo S, Nardini A, Gullo MAL, Mosca R. 1999. Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiology* **120**: 11–22.

Urli M, Porté AJ, Cochard H, Guengant Y, Burlett R, Delzon S. 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology* **33**: 672–683.

Wang R, Zhang L, Zhang S, Cai J, Tyree MT. 2014. Water relations of *Robinia pseudoacacia* L.: do vessels cavitate and refill diurnally or are R-shaped curves invalid in *Robinia*? *Plant, Cell & Environment*. doi: 10.1111/pce.12315.

Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook NM. 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant, Cell & Environment* **36**: 1938–1949.

Zufferey V, Cochard H, Ameglio T, Spring J-L, Viret O. 2011. Diurnal cycles of embolism formation and repair in petioles of grapevine (*Vitis vinifera* cv. Chasselas). *Journal of Experimental Botany* **62**: 3885–3894.

Zwieniecki M, Holbrook N. 1998. Diurnal variation in xylem hydraulic conductivity in white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). *Plant, Cell & Environment* **21**: 1173–1180.

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