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Editorial

Sap flow as a key trait in the understanding of plant hydraulic functioning

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Sap flow along the long distance water transport system in plants is essential in maintaining the hydraulic connection between the soil and the atmosphere. Studying the movement and dynamics of sap throughout a plant is hence key to progress our scientific knowledge about plant hydraulic function or dysfunction and growth in a given environment.

At the Ninth International Workshop on Sap Flow, held in Ghent, Belgium, from 4 to 7 June 2013, over 160 participants coming from 30 different countries discussed the current understanding of plant hydraulics and the significance of studies of sap flow in furthering our knowledge of plant water use and function under varying environmental conditions. These discussions dealt not only with measurement methodology, but focused largely on new insights into plant hydraulic function with a session specifically addressing cavitation as a limiting factor in the hydraulic pathway. Additionally, the importance and applicability of water transport modelling to deepen our understanding of plant hydraulics were considered and the use of sap flow for some practical applications was presented. From these sessions, 10 authors contributed research papers based on their talks for this special issue of *Tree Physiology* (Baert et al. 2015, Carrasco et al. 2015, Torres-Ruiz et al. 2015, Vandegehuchte et al. 2015, Vergeynst et al. 2015, Zeppel et al. 2014, Eller et al. 2015, Liesche et al. 2015, Paudel et al. 2015, Windt and Blümler 2015). This Editorial will highlight the major findings of these and other related papers, and of the invited Commentary (Rosner 2015).

Measurement methodology

Several advanced methods already exist to measure sap flow (Vandegehuchte and Steppe 2013) and assess plant water use. Nonetheless, efforts continue to be put into further optimization of the existing methods and into development of new approaches. Vandegehuchte et al. (2015), for instance, described an improved data analysis procedure for the heat ratio method (Burgess et al. 2001) by including a temperature correction, using the measured temperatures before application of the heat pulse, to correct for natural changes in stem temperature. Errors of up to 40% due to natural changes in stem temperature can thus easily be avoided. As with all sap flow techniques, accuracy is a key issue. The potential need for species-specific calibrations has been highlighted in the past (e.g., Steppe et al. 2010 and references therein), and calibration is thus encouraged in all sap flow studies. In cases where it is difficult, if not impossible, to perform species-specific calibrations (e.g., large trees), a healthy degree of skepticism should be maintained during data interpretation, but lack of sensor calibration should not outright lead to manuscript rejection, so long as the resulting error does not dominate the story told. A detailed Methods section addressing all technical issues related to sensor installation, sap flux density calculation and radial and azimuthal variability in the study trees is required in order to assess measurement reliability.

Given the dynamic nature of water movement throughout a plant, and its importance in plant hydraulic functioning, the study of tissue hydraulic capacitance and storage capacity is receiving

increasing interest. To this end, accurate determinations of water content are needed. Carrasco et al. (2015) presented a promising application of frequency domain reflectometry (FDR) in combination with point dendrometers and sap flow sensors to assess stem water storage use and refilling dynamics in the trunk of large subtropical tree species. Even though FDR might need species-specific calibration, the presented results were very encouraging, indicating the possibilities of this method to investigate water transport between sap conducting and storage tissues (Hao et al. 2013). Moreover, in combination with stem water potential measurements, continuous measurements of tissue water content may provide an easy way to determine tissue hydraulic capacitance.

Another technique employed to assess water content is the application of nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) (Windt et al. 2006, De Schepper et al. 2012). As NMR requires high-tech equipment, this method is mainly confined to laboratory studies. Windt and Blümler (2015), however, developed a small-scale portable NMR device to be used under field conditions. Based on a simple analysis of the relaxation signal, a way to measure dynamic changes in the absolute tissue water content, and potentially sap flow, was presented. Additionally, traditional analysis of the signal can provide information about the spatial distribution of xylem and phloem flows. Another way to assess phloem translocation speed is via the application of radioactive isotopes such as ^{11}C and ^{14}C as reported by Liesche et al. (2015). Their meta-analysis of experimental data on phloem transport speed in trees yielded average speeds of 56 cm h^{-1} for angiosperm trees and 22 cm h^{-1} for gymnosperm trees, with differences in sieve element anatomy being the main factor for the significantly slower carbohydrate transport speed in gymnosperm compared with angiosperm trees.

New insights into hydraulic plant functioning

Eller et al. (2015) reported on the evidence of foliar fog water uptake by the tropical cloud forest tree species *Drimys brasiliensis* Miers. (Winteraceae), showing that fog water uptake caused sap flow reversals of up to 26% of maximal daily transpiration (Eller et al. 2013). They showed how contrasting atmospheric and soil water conditions control diurnal, nocturnal and seasonal dynamics of sap flow in *D. brasiliensis*. This study contributes to the growing evidence that water movement throughout the soil–plant–atmosphere continuum (SPAC) can be reversed (Goldsmith 2013). It confirms that movement of sap throughout a plant occurs as a function of all available water sources causing changes in water potential gradients. Depending on these water potential gradients and the resistances in the hydraulic pathway, water will be distributed throughout the SPAC in a multidirectional way.

Clearly, hydraulic functioning of plants is a dynamic process, which is highly dependent on the environmental conditions in

which the plant thrives. As recent worldwide severe droughts have been associated with regional-scale forest mortality, and climate change is expected to intensify these drought events further, increasing attention is being placed on the understanding of the mechanisms of drought resistance and tree mortality (e.g., McDowell 2011, Anderegg et al. 2012, Zeppel et al. 2013). Future studies on physiological drought can profit from simultaneous measurements of sap flow and stem diameter variations on individual trees in order to assess global tree physiological responses to drought, as these measurements are relatively easy to obtain and reflect in an integrated way the hydraulic and carbon status of the tree.

Limitations in the hydraulic pathway

The possibility of occurrence of cavitation is an important aspect of the study of plant hydrology and is gaining increasing attention in sap flow research. As cavitation forms a major threat to sustained hydraulic transport, it is essential not only that we study the differences in embolism vulnerability and recovery within and between species, but also that we improve our understanding of how and why embolisms are formed and propagated in the xylem conduits. It has long been a mystery how xylem can operate under negative pressures, as gas bubble formation is expected in such a hydraulic system. These bubbles can expand to block conduits, form embolisms and disable the entire system. Schenk et al. (2015) explained that bubble formation through a pit membrane is not only dependent on the pressure gradient, pore radius, contact angle and surface tension as classically assumed by the Young–Laplace equation, but also by the shape of pores in the membrane. By taking this shape factor into account, much smaller bubbles than predicted can pass through the membranes. Based on this new paradigm, Schenk et al. (2015) proposed that most bubbles in the xylem sap are small and harmless, and that these nanobubbles do not pose a threat to plant hydraulic functioning. These authors argued that embolisms in the plant's hydraulic system may only form when an excessive number of bubbles are formed or when the water potential is too low.

But how should we measure cavitation? Most evidence for variations in the gas content of xylem conduits has been obtained by destructive hydraulic measurements, while emptying and refilling of xylem vessels has been shown in cryo-SEM studies. More recently, this has been confirmed non-destructively via MRI and high-resolution X-ray computed microtomography (μCT). While in situ measurements of stem water content and active conductive area may provide an estimate of the proportion of cavitated xylem conduits, acoustic emission (AE) analysis seems a promising approach to determine the occurrence of cavitation events (Rosner et al. 2006). Vergeynst et al. (2015) reported a new type of vulnerability curve based on continuous AE measurements, radial diameter shrinkage and

gravimetric water loss. With this method the two consequences of cavitation can be quantified, as it allows assessment of the loss in hydraulic conductivity and the gain in hydraulic capacitance during the cavitation phase. Rosner (2015) highlighted that the construction of vulnerability curves based on AE measurements has been proven to work well in conifers, but that it has always been a challenge in angiosperm wood, with the main difficulty defining the 100% conductivity loss. Rosner (2015) commented that the approach taken by Vergeynst et al. (2015) is reliable because of the strong similarity in pattern between visually (on μ CT images) and acoustically detected cavitations, and that the new method has high potential to become a benchmark. In their paper, Torres-Ruiz et al. (2015) reported on the strong hydraulic segmentation in olive plants, with protection of the stem hydraulic function by a faster hydraulic failure in roots and leaves. These losses in hydraulic conductivity in the distal plant organs also controlled stomatal behaviour more than increased levels in abscisic acid during drought stress, while the combined role of hydraulic and chemical signals remained unclear during recovery.

Modelling plant-water transport

Process-based plant models are necessary to build a mechanistic understanding of a plant's water and carbon balance. As processes such as xylem and phloem flows, elastic cell expansion and irreversible growth are subject to the laws of physics, they can be mechanistically described in relation to plant biology. Models are also essential to integrate tree functioning with internal and external factors offering the benefit of predicting plant responses to changing environmental conditions (Fonti and Jansen 2012). In their paper, Paudel et al. (2015) simulated tree transpiration and dynamic water storage from sap flow responses, photosynthetic active radiation and vapour pressure deficit. While many existing models focus only on water flow (e.g., Paudel et al. 2015), increasing effort is being put into the integration of phloem flow in plant models, given the important coupling between hydraulic processes and the transport and allocation of carbohydrates (Hölttä et al. 2009, De Schepper and Steppe 2010).

Implementation and application of process-based plant models has greatly advanced our knowledge on plant hydraulic functioning and growth. As such, models have allowed researchers, among other things, to better interpretation of stomatal regulation (Zweifel et al. 2007), stem CO₂ efflux (Hölttä and Kolari 2009), stem diameter variations and radial stem growth (Zweifel et al. 2001, Steppe et al. 2006), to simulate physiological variables which are difficult to measure such as osmotic and turgor pressure in the phloem and phloem sugar transport rates (De Schepper and Steppe 2010), to link phloem function to structure (Hölttä et al. 2009) and to derive phloem-generated turgor signals (Mencuccini et al. 2013).

However, many challenges remain in optimizing existing models and incorporating the additional processes necessary to describe specific hydraulic functions. Baert et al. (2015), for instance, improved the representation of some processes represented in the model of Steppe et al. (2006). These authors showed that implementing dynamic functions to describe xylem axial hydraulic resistance and the resistance between xylem and storage tissues instead of the previously applied constant parameters greatly improved model performance for drought stressed conditions. Zeppel et al. (2014) reviewed the consequences of nocturnal water loss. Accounting for the effect of night-time transpiration on the processing of sap flux signals may substantially increase estimated daily water uptake of trees (e.g., by 30% in mature Norway spruce, Ward et al. 2008). Zeppel et al. (2014) warned of underestimation of total plant water use and inaccurate estimation of ecosystem level water balance when models assume that night-time stomatal conductance is zero. Other examples of opportunities to improve process-based plant models, especially to simulate hydraulic stress, are the incorporation of the dynamics of cavitation, phytohormone transport and canopy water uptake.

Future challenges

When installing sap flow sensor needles, flow obstruction in the conductive tissue occurs. This wound effect is known to cause underestimations for heat pulse methods (Burgess et al. 2001, Green et al. 2009, Vandegehuchte and Steppe 2012), but it is also likely to affect other needle-based methods. While wound correction factors have so far only been based on wound width, the underestimation will also be dependent on the thermal properties of the sapwood. Wound effects may also progress with time (Wiedemann et al. 2013), potentially limiting the utility of empirically based sensors for addressing longer-term processes, such as comparison among treatments or seasons if the same sensors are followed over time. However, by relativizing the values of sap flow of each species to the measured maximum, and by limiting the measurements to a short period after installation, before the wound effect takes hold, at least the rate of change with respect to changes in environmental variables can be compared among species. More in-depth studies are thus needed to investigate differences in wound effect and progression rates in individuals of a given species, and between species. The application of NMR or μ CT seems promising as these methods allow characterization of the flow obstruction zone accurately.

Besides improvement of sensor accuracy, it is also of crucial importance to adapt the sensors to different plant sizes. Both heat pulse and continuous sap flow methods have already been down-scaled (e.g., van Bavel and van Bavel 2005, Clearwater et al. 2009, Hanssens et al. 2013). A future important step would be to adapt the methods enabling water content measurements to smaller scales as well, as this could greatly improve our

understanding of the vascular fluxes at the scale of fruits and leaves. On the other hand, further improvement in the upscaling of sap flow measurements is necessary, as in many studies the question of how to translate point measurements to an entire stand or ecosystem remains difficult, with upscaled estimates often affected by the sensor-to-stand scaling procedure (Oishi et al. 2008).

As clearly put forward during the workshop, further research focusing on the hydraulic as well as the carbon balance in plants will be necessary to unravel the mechanisms playing a role during hydraulic stress events. Given the role of transport and remobilization of non-structural carbohydrates, not only the hydraulic limitations in the xylem, but also in the phloem must be investigated. On one hand, detailed studies at the scale of plants and tissues are required, but on the other hand, a continental and even a global approach is needed. As drought events cannot easily be confined to laboratory conditions, it is of importance to try to determine the different drought responses between and within species under field conditions at the relevant spatial and temporal scales. In the late 1980s, Kramer advocated the need to consider plant responses to drying forces of both soil and atmosphere in field conditions to avoid rushing into employing responses observed in the laboratory to explain what is seen in the field (Kramer 1988). Measuring sap flow in combination with other easily measurable variables, such as stem diameter variations, holds promise of addressing this challenge, as these continuous in situ measurements in the field can greatly assist us to properly link tree physiology to ecophysiology.

To integrate and deepen our knowledge of plant hydraulic functioning, process-based plant models are likely to become increasingly important. Despite the large improvements in plant modelling during the past few decades, many models still have difficulties in describing hydraulic stress responses. The implementation of new mechanisms unveiled by experimental studies will allow further improvement of these models. This will require a close collaboration between plant physiologists and modellers and an approach in which processes at cell, tissue, organ and plant level will be integrated.

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