Commentary

Modelling the outside-xylem hydraulic conductance: towards a new understanding of leaf water relations

One of the most tantalizing unresolved questions in plant hydraulics is: where does water evaporate within the leaf? While several studies have explored experimental routes to answer this question (e.g. Sheriff & Meidner 1974; Farquhar & Raschke 1978), none have been able to provide a clear answer. In this issue of *Plant, Cell & Environment*, Tom Buckley has presented a new mathematical model incorporating anatomical and biophysical parameters to estimate hydraulic conductance in the apoplast, symplast and gas phase pathways outside the leaf xylem (Buckley 2014). Buckley reports that the apoplastic route was the most conductive flow path for water, but that gas phase pathways can represent a substantial portion of water movement, especially when high temperature gradients exist within the leaves (from high temperature at the leaf veins to lower temperature values at the epidermis), a result previously described in another modelling paper earlier this year (Rockwell *et al*. 2014). Buckley's model provides, for the first time, equations to estimate outside-xylem hydraulic conductance based on real leaf anatomy and found that the proportion of total water movement in the different pathways was strongly dependent on mesophyll anatomy. This new mathematical model has the potential to finally answer the long-standing question of where water evaporates in leaves of a given species.

Knowing the site of water evaporation within the leaf is crucial to our understanding of water movement outside the xylem. Leaf hydraulic conductance (K_{leaf}) is a measure of the capacity of water to move through the leaf and a major determinant of plant growth (Sack & Holbrook 2006). Unlike in stems, and just like in roots, water moves through both xylem and living cells outside the xylem. Understanding water flow through the outside-xylem pathways remains the largest challenge in the field of leaf hydraulics. Three main hypotheses of where most of the water evaporates from within the leaf have been proposed based on empirical data and models (Fig. 1a,b,c): (1) water mainly evaporates from the liquid phase once it reaches the substomatal cavities and diffuses through stomata (e.g. Sheriff & Meidner 1974; Tyree & Yianoulis 1980; Fig. 1a); (2) water flows through the bundle sheath cells and principally evaporates there (Boyer 1985; Fig. 1b); and (3) water evaporates from the mesophyll and epidermal cells, with an appreciable part evaporating near the stomata (Farquhar & Raschke 1978; Fig. 1c).

Based on a novel analytical framework, Buckley (2014) offers a new view of where water evaporates, partially reconciling the previous hypotheses described above and in accordance with what Rockwell *et al*. (2014) proposed earlier

this year (Fig. 1d). According to this new view, water will move in parallel through both liquid and gas phases until it reaches the stomata. In this model, the intercellular airspaces in the leaf are viewed as conduits for water transport, and thus, after moving through the bundle sheath cells, water can either continue flowing as liquid through the apoplastic and symplastic pathways, evaporating near stomata, or it can evaporate at the bundle sheath, and diffuse as gas until it reaches stomata (Fig. 1d). Because both liquid and gas phases share the same low water potential sink (airspaces near the stomata), they also share the same water potential gradient along the way, thus no evaporation would occur from mesophyll cells along the liquid path because the liquid water would be at the same water potential as the gas phase in the adjacent intercellular airspaces. The site of evaporation in the leaf would thus depend on where the bulk of the flow occurs, whether as vapour diffusion from near the bundle sheath, or liquid phase through the mesophyll and epidermis, evaporating at the most distal sites along the pathway. Buckley's model allows us to determine where evaporation would occur, given the knowledge of species' leaf anatomy. Because hydraulic conductance through both liquid and vapour phase pathways was highly dependent on leaf anatomy and temperature gradients within the leaf, different species could exhibit different evaporation sites. For example, species with very thick cell walls and low temperature gradients within the leaf could potentially have the bulk of its evaporation take place close to the stomata (such as shown in Fig. 1a). That would be expected because apoplastic conductance would be high and evaporation potential would be similar throughout the leaf. On the other hand, species with very thin cell walls and large temperature gradients could potentially have the bulk of its evaporation take place at the bundle sheath (such as shown in Fig. 1b). That would be expected because apoplastic conductance would be low and evaporation would be higher within the leaf, where the temperature is highest. Testing of this model against empirical data should show very promising results towards a better understanding of water movement outside the xylem.

Buckley's model can also give us great insight into the possible causes of leaf hydraulic decline with dehydration. Indeed, K_{leaf} has been shown to decline with dehydration, with drought-sensitive species usually declining rapidly at mild water stress (Sack & Holbrook 2006; Scoffoni *et al*. 2014). While the decline of hydraulic conductance inside the xylem (K_x) with dehydration is fairly well understood (cavitation – embolizing conduits – and/or xylem cell wall collapse), few studies have looked at how dehydration impacts outside-xylem hydraulic conductance (K_{ox}) and how this would affect K_{leaf}. Knowing the impact of K_{ox} on K_{leaf} decline

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(a) Water mainly evaporates near stomata

(b) Water mainly evaporates at the bundle sheath

(c) Water evaporates everywhere along the liquid path

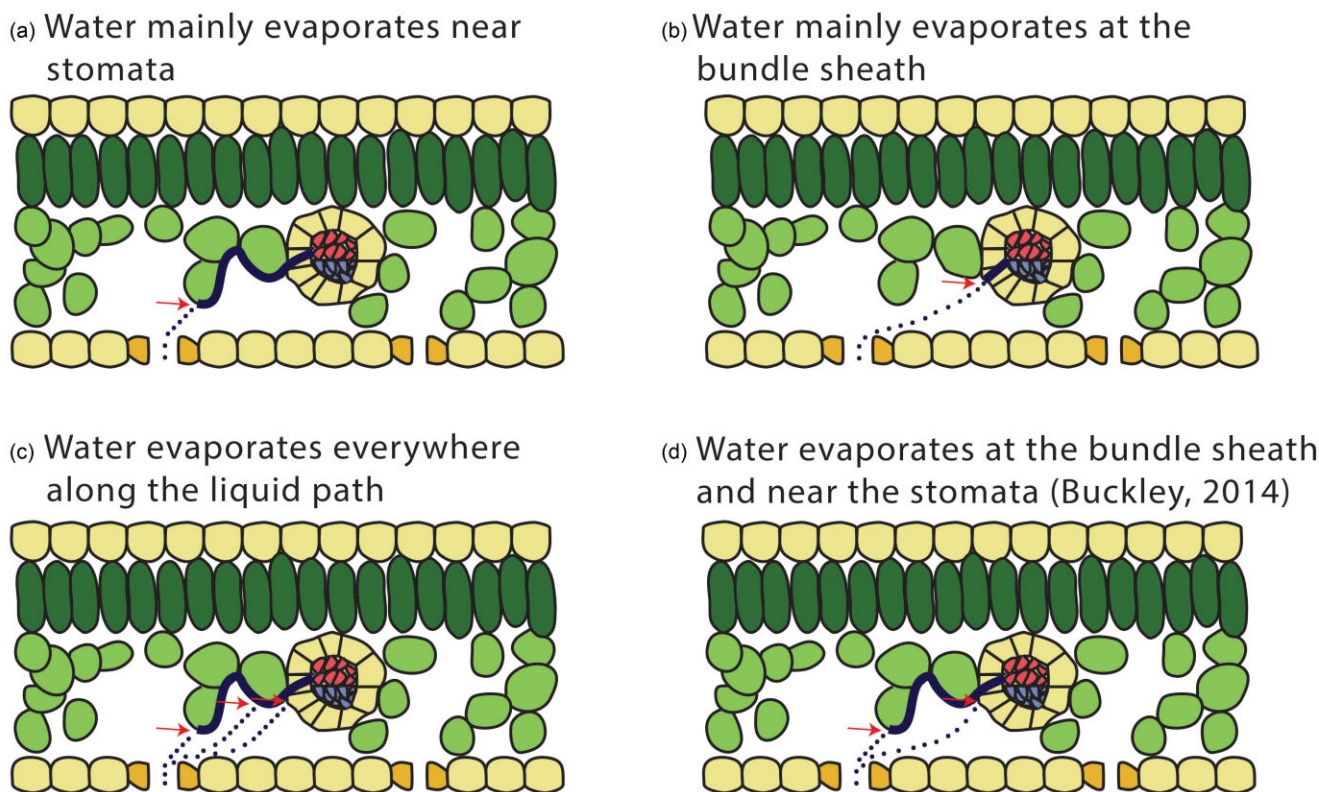**(d) Water evaporates at the bundle sheath and near the stomata (Buckley, 2014)**

Figure 1. Current theories of water movement outside the xylem to the site(s) of evaporation. (a) Water flows from the xylem, through the bundle sheath, then moves in liquid form apoplastically and/or symplastically through the mesophyll until it mainly evaporates near the stomata and diffuses out of the leaf (Sheriff & Meidner 1974; Tyree & Yianoulis 1980). (b) Water flows from the xylem through the bundle sheath cells where it mainly evaporates and diffuses out of the stomata (Boyer 1985). (c) Water flows from the xylem through the bundle sheath, then moves in liquid form apoplastically and/or symplastically through the mesophyll and evaporates along the way, with the bulk of the evaporation occurring near the stomata (Farquhar & Raschke 1978). (d) Water flows from the xylem through the bundle sheath, then water both evaporates at the bundle sheath and diffuses through stomata, as well as moving in liquid form apoplastically and/or symplastically through the mesophyll and evaporating near the stomata (Buckley 2014; Rockwell *et al.* 2014). The site where the bulk of the evaporation takes place will depend on the distribution of conductance among apoplastic, symplastic and gas phase pathways, which can be determined for a given species by parameterizing Buckley's model with species anatomical traits. Red arrows indicate the sites of evaporation in the leaf.

is of special interest as recently it has been suggested that cavitation might not have as strong an effect as was previously thought in stems (Delzon & Cochard 2014) or leaves (Scoffoni & Sack 2014). Indeed, simulations using a spatially explicit model of a leaf showed that because of the water potential gradients that develop in the transpiring leaf, the most negative water potentials would be at the distal (mesophyll) end of the hydraulic pathway, and xylem pressures would rarely reach pressures that would induce air seeding and cavitation (Scoffoni *et al.* 2014). Thus, the bulk of K_{leaf} decline with dehydration might be explained by changes in the outside-xylem pathways. Recent studies have shown that aquaporin deactivation, loss of cell turgor and/or loss of cell connectivity could have an important impact on K_{leaf} decline (e.g. Koroleva *et al.* 1997; Kim & Steudle 2007; Scoffoni *et al.*, 2014). The analytical framework proposed by Buckley (2014) points towards an integrative view of these possible factors. Depending on where the bulk of the flow occurs in the leaf, aquaporin deactivation (changing cell permeability) could have more of an impact on K_{leaf} than loss of cell connectivity

(which especially influences apoplastic pathways). The location of the bulk of the flow outside the xylem might thus also change as the leaf dehydrates. Anatomical traits of dehydrated leaves are needed to parameterize the model and test against empirical data to provide clearer answers.

Buckley's model also presents an additional mechanism for the well-documented increases of K_{leaf} with temperature and irradiance. Both responses of K_{leaf} to temperature and light have typically been attributed to the effect of aquaporins across cell membranes as well as to the change in the viscosity of xylem water in the case of temperature (e.g. Sack *et al.* 2004; Cochard *et al.* 2007). Cochard *et al.* (2007) suggested two distinct routes of water movement outside the xylem under high and low irradiance: (1) water moves both along cell walls and across cell membranes when aquaporins are activated under high irradiance; and (2) water only moves apoplastically when aquaporins are deactivated under low irradiance. Buckley's results, along with those of Rockwell *et al.* (2014), suggest a third possible route, that of vapour phase. Indeed, vapour phase pathways would be greatly

increased under higher temperatures and/or higher irradiance that might heat the inner mesophyll, as the driving force is a concentration gradient rather than a pressure gradient. Additionally, depending on the distribution of conductance outside the xylem, aquaporin deactivation could have more or less of an impact on K_{ox}. Further simulations and testing against empirical data will provide clearer answers.

Finally, it is to be noted that, as of now, Buckley's model does not include flow through bundle sheath cells. Past studies have shown a great impact on K_{leaf} decline of both aquaporin deactivation and loss of cell turgor in the bundle sheath cells (e.g. Koroleva *et al*. 1997; Kim & Steudle 2007; Prado *et al*. 2013). Inclusion of water movement through the bundle sheath would not change any of the resulting theory described earlier, but could drastically change the range of proportion of conductance through the apoplast, symplast and gas phase pathways presented in Buckley (2014). Indeed, water has to move through bundle sheath cells to access any of the routes to the mesophyll and sites of evaporation, and could thus present an important bottleneck in water movement outside the xylem.

Future studies in leaf hydraulics will increasingly focus on the effect of outside-xylem pathways on K_{leaf}. The insights and analytical framework in Buckley (2014) have provided us with important tools towards a better understanding of our field.

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