

### Appendix S3. Mesophyll osmotic water permeability

The water flux,  $J$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ), across a membrane with osmotic water permeability  $P_m$  ( $\text{m s}^{-1}$ ) for a transmembrane water potential gradient  $\Delta\psi$  (Pa) is given by (Buckley, 2015)

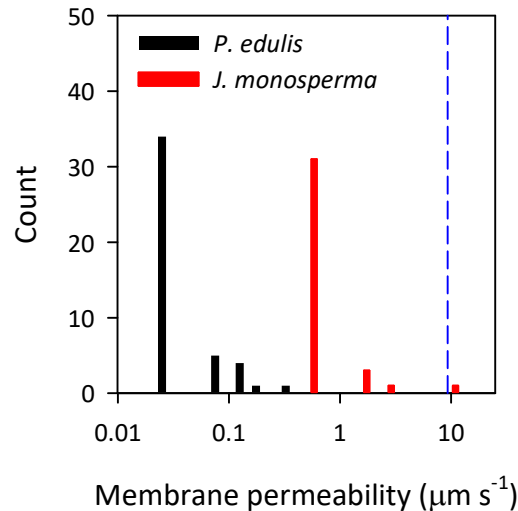
$$(S5) \quad J = \frac{P_m}{RT} \Delta\psi .$$

Assuming that these membranes support a total water flow equal to the transpiration rate,  $E$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ), and that the total area of mesophyll cells is a multiple,  $s_{\text{mes}}$  ( $\text{m}^2 \text{m}^{-2}$ ), of the associated leaf area, then  $J = E/s_{\text{mes}}$ . Applying this to Eq. S5 and solving for  $P_m$  gives

$$(S6) \quad P_m = \frac{ERT}{s_{\text{mes}} \Delta\psi} .$$

We are unaware of published measurements of  $s_{\text{mes}}$  in *P. edulis*, but  $s_{\text{mes}}$  varied from 14.4 to 40  $\text{m}^2 \text{m}^{-2}$  across 15 diverse species in a study by Tomas et al. (2013). We adopt  $s_{\text{mes}} = 10 \text{m}^2 \text{m}^{-2}$  to ensure the resulting estimate of  $P_m$  is not an underestimate due to uncertainty in this parameter. Applying Eq. S6 to the data of Cernusak et al. (2018) indicates that, to sustain a symplastic water potential of -2 MPa while water flows across the cell membranes to an apoplastic space with water potential given by Eq. S1 would require  $P_m$  as low as  $1.7 \cdot 10^{-9} \text{m s}^{-1}$ , or  $0.0017 \mu\text{m s}^{-1}$ , for *P. edulis*, and as low as  $9.8 \cdot 10^{-8} \text{m s}^{-1}$ , or  $0.0098 \mu\text{m s}^{-1}$ , for *J. monosperma* (Appendix S3a). For *P. edulis*, 25% of measurements indicate  $P_m$  at least as small as  $0.0083 \mu\text{m s}^{-1}$ ; for *J. monosperma*, 25% of measurements indicated  $P_m < 0.099 \mu\text{m s}^{-1}$ . By comparison, mean values of  $P_m$  previously reported for mesophyll protoplasts are orders of magnitude larger (e.g., 5–25  $\mu\text{m s}^{-1}$ ; Shatil-Cohen et al., 2013).

If only a small number of cells supported the transpirational flow across membranes, then the  $P_m$  in those cells could be quite large, and in the range of values typically observed; however, that would require the corollary assumption that all other cells did *not* support transmembrane flow (despite the occurrence of a large water potential gradient across the membrane), which in turn would require an even lower value of  $P_m$  for the latter cells than estimated above.



Appendix S3a. Distributions of inferred cell membrane permeabilities needed to reconcile low inferred water potentials at the cell wall evaporating site with measured water vapor fluxes, in the data from Cernusak et al. (2018), for *Pinus edulis* (black bars) and *Juniperus monosperma* (red bars). Each vertical bar represents the number of data points in a discrete "bin" centered at the values shown (e.g., 34 points for *P. edulis* were in the lowest-permeability bin, centered at 0.025  $\mu\text{m s}^{-1}$ ; some individual points had permeabilities well below that value, down to 0.0017  $\mu\text{m s}^{-1}$ ). The blue dashed line indicates a value of 9.3  $\mu\text{m s}^{-1}$ , the mean value among 80 mesophyll cells measured by Shatil-Cohen et al. (2011) in *Arabidopsis thaliana*.

## References

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