Supplemental File S1

Derivation of expressions for flow area, pathlength corrections, grid areas, grid pathlengths and volume averaging basis adjustment

To accompany "How does leaf anatomy influence water transport outside the xylem?" by TN Buckley et al.

Flow areas per unit bulk area, γ

For flow across membranes, the potential area for transport may be greater than the simple cross-sectional area, or bulk area. For example, the curved surfaces of mesophyll cells present a greater area of membrane for transport than the simple projected areas of those cells in the direction of flow. However, the effective area is also reduced in proportion to the connectivity of mesophyll cells (the fraction of total surface area that is in contact betweenadjacent cells; f_c), and the complement of the tissue porosity (p_p). The correction factors (γ) are the ratios of actual contacting surface area to projected cross sectional area. For vertical transmembrane flow in palisade tissue, this is

(S1) $$\gamma_{m,pv} = (1 - p_p) f_c \frac{\frac{1}{2} 4\pi r_p^2}{\pi r_p^2} = 2(1 - p_p) f_c$$

The numerator is one-half of the surface area of a sphere, which is also the surface area of the curved end of a capsule. The denominator is the projected cross-sectional area of the cell. An identical expression also arises for both vertical and horizontal transmembrane flow in spongy tissues:

(S2) $$\gamma_{m,sv} = \gamma_{m,sh} = 2(1 - p_s) f_c$$

The surface area of a capsule is $4 \cdot \pi \cdot r_p^2 + 2 \cdot \pi \cdot r_p(h_p - 2r_p)$ and the cross sectional area along the long axis is $\pi \cdot r_p^2 + 2r_p(h_p - 2r_p)$. Therefore, for horizontal transmembrane flow in palisade tissue, (S3) $$\gamma_{m,ph} = \left(1 - p_p\right) f_c \frac{\frac{1}{2}\left(4\pi r_p^2 + 2\pi r_p \left(h_p - 2r_p\right)\right)}{\pi r_p^2 + 2r_p\left(h_p - 2r_p\right)} = \frac{\pi\left(1 - p_p\right) f_c h_p}{2h_p - \left(4 - \pi\right) r_p}$$

All other cell types are modeled as rectangular boxes with zero tissue porosity, so their ratios of projected and actual transmembrane flow areas are simply unity.

For apoplastic flow paths, the total area available for flow is approximately the product of cell circumference, cell wall thickness and cell wall porosity, whereas the bulk area is the cell cross sectional area divided by the complement of tissue porosity. For vertical apoplastic flow in the palisade, this gives

(S4) $$\gamma_{a,pv} = \frac{t_{ap} \cdot 2\pi r_p \cdot p_a}{\pi r_p^2 \big/ \left(1 - p_p\right)} = \frac{2 p_a \left(1 - p_p\right) t_{ap}}{r_p}$$

For horizontal apoplastic flow in the palisade, this gives

(S5) $$\gamma_{a,ph} = \frac{p_a \left(1 - p_p\right) t_{ap} \left(2\pi r_p + 2h_p\right)}{\pi r_p^2 + 2r_p\left(h_p - 2r_p\right)}$$

For horizontal or vertical flow in the spongy mesophyll, the result is analogous to $\gamma_{a,pv}$:

(S6) $$\gamma_{a,sv} = \gamma_{a,sh} = \frac{2 p_a \left(1 - p_s\right) t_{as}}{r_s}$$

We modeled epidermal cells as rectangular boxes with square bases of width w_e and height h_e. The area correction for vertical apoplastic flow into the epidermis is thus

(S7) $$\gamma_{a,ev} = \frac{p_a 4 w_e t_{ae}}{w_e^2} = \frac{4 p_a t_{ae}}{w_e}$$

For horizontal flow, the result is

(S8) $$\gamma_{a,eh} = \frac{p_a\left(2w_e + 2h_e\right)t_{ae}}{w_e h_e} = 2p_a t_{ae}\left(\frac{1}{h_e} + \frac{1}{w_e}\right)$$

We modeled bundle sheath and BSE cells as cubes with width w_b and w_x, respectively, which gives results analogous to $\gamma_{a,ev}$:

(S9) $$\gamma_{a,xv} = \gamma_{a,xh} = \frac{4p_a t_{ax}}{w_x}\text{, and}$$

(S10) $$\gamma_{a,b} = \frac{4p_a t_{ab}}{w_b}.$$

Finally, for gas flow, the area correction is simply the tissue porosity: $\gamma_{g,p} = p_p$ and $\gamma_{g,s} = p_s$ for palisade and spongy mesophyll, and zero for all other tissues.

Flow pathlengths per unit bulk pathlength, β

For apoplastic flow in spongy mesophyll and horizontal apoplastic flow in palisade mesophyll, the direct route across a cell is twice its radius, whereas the minimum apoplastic route is half of the cell circumference, or π times its radius, corrected for mesophyll connectivity (f_c). The terms for radius cancel out, giving

(S11) $$\beta_{a,sh} = \beta_{a,sv} = \beta_{a,ph} = \tfrac{1}{2}\left(1 - f_c\right)\pi$$

For vertical apoplastic flow in palisade mesophyll, the direct and apoplastic routes are both longer than for horizontal flow by the cell height h_p minus twice the radius, which gives

(S12) $$\beta_{a,pv} = \frac{(1-f_c)\pi r_p + (h_p - 2r_p)}{2r_p + (h_p - 2r_p)} = 1 + ((1-f_c)\pi - 2)r_p / h_p$$

The actual and direct flow paths are equivalent for all other tissues and modes of flow, giving β = 1.

Grid areas (a) and pathlengths (l)

The grid is a set of 744 tissue bands delineated by 25 planes parallel to the epidermis, and 31 concentric cylinders centered on a vertical axis located at the center of the areole. The upper- and lower-most planes coincide with the upper and lower leaf surfaces, respectively, thus defining 24 "rows" of tissue bands (indicated below with subscripted indices *i*). The outermost cylinder coindicides with the lateral midpoint of the nearest minor vein, thus defining 31 "columns" of tissue bands (indicated below with indices *j*).

With three exceptions, the vertical and radial thicknesses of these tissue bands are identical to one another. Two of these exceptions are the uppermost and lowermost rows (i = 1 and 31, respectively), whose thicknesses are defined by the measured upper and lower epidermis thicknesses, respectively. All other rows are defined as 1/29th of the remaining leaf thickness (equal to the sum of measured palisade and spongy mesophyll tissue thicknesses, t_p and t_s). The third exception, which applies only in heterobaric species (those possessing bundle sheath extensions) is the outermost column (j = 1), whose thickness is defined as one-half of the measured bundle sheath extension width. In these species, the widths of all other tissue columns are equal to 1/23rd of the difference between areole radius (r_{areole}) and BSE half-width ($w_{\mathrm{x,tot}}/2$). In homobaric species (which lack bundle sheath extensions), all columns have the same width, which is 1/24th of the areole radius. These three exceptions ensure that the volumes, horizontal areas and flow pathlengths involving the epidermis and/or BSEs are appropriate to the actual tissue dimensions. (Further corrections are required to accommodate the modeled geometry of the bundle sheath; these are described in the next section.)

The area for horizontal flow between tissue bands *j* and *j*+1 with thickness t_i ($t_i = t_e$ for epidermal rows or $(t_p + t_s)/29$ otherwise) is equal to the product of t_i and the circumference of the outer cylinder bounding the band at *j*+1. For heterobaric species, this area is

$$\text{(S13)} \quad a_{h,i}[j;\, j+1] = t_i \cdot 2\pi\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)\left(1 - (j-1)/23\right)$$

and for homobaric species, this area is

$$\text{(S14)} \quad a_{h,i}[j;\, j+1] = t_i \cdot 2\pi r_{areole}\left(1 - j/24\right)$$

The area for vertical flow between bands at column *j* and rows *i* and *i*+1 is equal to the vertical projected area of the band at column *j*. This equals the difference between the areas of circles defined by the outer and inner radial boundaries of column *j*. For the outermost column (*j* = 1) in heterobaric species, this area is

$$\text{(S15)} \quad a_{v,j=1} = \pi r_{areole}^2 - \pi\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)^2$$

For all other columns in heterobaric species, the area is

$$\text{(S16)} \quad \begin{aligned} a_{v,j>1} &= \pi\left[\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)\left(1 - (j-1)/23\right)\right]^2 - \pi\left[\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)\left(1 - j/23\right)\right]^2 \\ &= \pi\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)^2 (47 - 2j)/529 \end{aligned}$$

For all columns in homobaric species, the area is

$$\text{(S17)} \quad a_{v,j} = \pi r_{areole}^2 (49 - 2j)/576$$

The direct flow pathlengths between adjacent bands are computed as the distances between the vertical and radial midpoints of those bands. Thus, the direct pathlength between the upper epidermis (*i* = 1) and the row of tissue bands directly below it (*i* = 2) equals one-half of the upper epidermis thickness (t_{eu}) plus one-half of the thickness of one non-epidermis band, which as described above is 1/29th of the sum of palisade and spongy tissue thicknesses. This flow path is

(S18) $$l_v[i=1;i=2]=\tfrac{1}{2}t_{eu}+\tfrac{1}{29}\left(t_p+t_s\right)$$

Similarly, the direct flow pathlength between rows 30 and 31 (the lower epidermis) is

(S19) $$l_v[i=30;i=31]=\tfrac{1}{2}t_{el}+\tfrac{1}{29}\left(t_p+t_s\right)$$

The direct vertical flow pathlengths between all other rows is simply

(S20) $$l_{v,2\le i\le 30}=\tfrac{1}{29}\left(t_p+t_s\right)$$

The direct horizontal flow pathlength between the outermost tissue band column (j = 1) and the adjacent column (j = 2) differs for heterobaric and homobaric species. For heterobaric species, the value is one-half of the bundle sheath extension half-width plus one-half of 1/23 of the remainder of the areole radius:

(S21) $$l_h[j=1;j=2]=\tfrac{1}{4}w_{x,tot}+\tfrac{1}{46}\left(r_{areole}-\tfrac{1}{2}w_{x,tot}\right)$$

For connections between all other adjacent columns in heterobaric species, the value is

(S22) $$l_{h,j\ge 2}=\tfrac{1}{23}\left(r_{areole}-\tfrac{1}{2}w_{x,tot}\right)$$

For homobaric species, the direct horizontal flow pathlength between any two adjacent columns is simply 1/24th of the areole radius:

(S23) $$l_h=r_{areole}/24$$

Estimating the number of grid rows for each tissue type

We estimated the number of grid rows for different tissue types based on measured tissue thicknesses, as follows. The number of bundle sheath rows (n_{bs}) was the greater of 1 and the quantity $29 \cdot h_{bs}/(t_p + t_s)$, rounded to the nearest whole number. This recognises that the palisade and spongy mesophyll tissue thicknesses combined ($t_p + t_s$) occupy 29 grid rows in total. The number of rows between the BS and the upper epidermis, n_{xu}, was computed as $(29 - n_{bs}) \cdot h_{xu}/(h_{xu} + h_{xl})$ (rounded to the nearest whole number), where h_{xu} and h_{xl} are the distances from the bundle sheath to the upper and lower epidermis, respectively; the number of rows between the BS and the lower epidermis, n_{xl}, was then $29 - n_{bs} - n_{xu}$. The number of palisade rows (n_p) was $29 \cdot t_p/(t_p + t_s)$, rounded to the nearest whole number, and the numer of spongy mesophyll rows (n_s) was $29 - n_p$.

Corrections to account for bundle sheath geometry

We modeled the bundle sheath as the space between the radial faces of two torus-like objects: one is actually an elliptic torus, and the other is a similar solid of revolution that is nested within the elliptic torus but has dimensions such that the distance from its surface to that of the elliptic torus is everywhere identical. The elliptic torus represents the outer face of the BS (the face farther from the xylem), the smaller (inner) torus-like object is the inner face, and the constant distance between the two faces represents the constant thickness of the BS itself. The outer face contacts mesophyll and BSE tissues. However, because the BS is represented in the grid as simply a stack of cylindrical tissue bands in the outermost column of tissue bands, the total area of the BS is not accurately represented by the grid areas computed as described in the preceding section. We therefore corrected the values for BS bulk conductivity applied to the grid in such a way that the total hydraulic conductance out of the BS accurately reflects the toroidal model described above. In this section, we describe how the relevant areas and corrections were calculated.

The area of the inner and outer faces of the BS can be computed using Pappus' Centroid Theorem, which states that the surface area of a surface of revolution created by revolving a curve about an axis is equal to the product of the arc length of the curve and the distance travelled during the revolution by the curve's centroid (the point coinciding with the geometric average of all points in the curve). To compute these values, we therefore require the appropriate arc lengths, centroids and radii of revolution. The major radius of both tori is equal to the areole radius. The vertical radius of the ellipse (the "tube") for the outer torus is one-half of the measured height of the bundle sheath (h_{bs}), and the horizontal radius of that ellipse is computed from h_{bs} and the bundle sheath perimeter (p_{bs}) as $p_{bs}/\pi - h_{bs}/2$. The two radii of the ellipse for the inner torus are smaller than the analogous values for the outer torus by an amount equal to the measured bundle sheath cell thickness (t_{bs}).

The arc length for the outer face is thus simply $p_{bs}/2$, and the arc length for the inner face is $\pi((p_{bs}/\pi - h_{bs}/2 - t_{bs}) + (h_{bs}/2 - t_{bs}))/2 = \pi(p_{bs}/\pi - 2\cdot t_{bs})/2 = p_{bs}/2 - \pi\cdot t_{bs}$. It is easily shown that the centroid for the outer face is located at a distance $4(p_{bs}/\pi - h_{bs}/2)/(3\pi)$ from the edge of the areole, and the centroid for the inner face is located at a distance $4(p_{bs}/\pi - h_{bs}/2 - t_{bs}))/(3\pi)$. The distance travelled by these centroids during revolution is 2π times the difference between r_{areole} and each of these values. Thus, the area of the outer face is

$$\text{(S24)} \quad a_{bs,out} = \left[\frac{p_{bs}}{2}\right] \cdot \left[2\pi\left(r_{areole} - \frac{4}{3\pi}\left(\frac{p_{bs}}{\pi} - \frac{h_{bs}}{2}\right)\right)\right]$$

and the area of the inner face is

$$\text{(S25)} \quad a_{bs,in} = \left[\frac{p_{bs}}{2} - \pi t_{bs}\right] \cdot \left[2\pi\left(r_{areole} - \frac{4}{3\pi}\left(\frac{p_{bs}}{\pi} - \frac{h_{bs}}{2} - t_{bs}\right)\right)\right]$$

The total computed grid area for contact between the BS and mesophyll in heterobaric species is n_{bs} times the horizontal projected area for contact between the outermost column and the adjacent column, which from Eqn S13 is

(S26) $$a_{grid,bs-mes} = n_{bs} \cdot \frac{(t_p + t_s)}{29} \cdot 2\pi\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)$$

For homobaric species, this area (from Eqn S14) is

(S27) $$a_{grid,bs-mes} = n_{bs} \cdot \frac{(t_p + t_s)}{29} \cdot 2\pi r_{areole}\left(\frac{23}{24}\right)$$

The total computed grid area for BS to BSE contact in heterobaric species is equal to twice the vertical projected area of a tissue band in the outermost column, which from Eqn S15 is

(S28) $$a_{grid,bs-bse} = 2\left(\pi r_{areole}^2 - \pi\left(r_{areole} - \tfrac{1}{2} w_{x,tot}\right)^2\right)$$

For homobaric species, from Eqn S17 this area is

(S29) $$a_{grid,bs-mes,vert} = 2\pi r_{areole}^2 \, 47/576$$

We assumed that the fraction of the total outer BS surface area in contact with the BSEs was equal to the BSE width divided by one-half of the BS perimeter ($2 \cdot w_{x,tot}/p_{bs}$), so that the total BS-BSE surface area was $2 \cdot w_{x,tot} \cdot a_{bs,out}/p_{bs}$. Thus, when calculating conductances for vertical transport between BS and BSE nodes in heterobaric species, the bulk flow area computed from Eqn x was corrected by the ratio

(S30) $$\frac{(2w_{x,tot}/p_{bs})a_{bs,out}}{a_{grid,bs-bse}}$$

For conductances for horizontal transport between BS and mesophyll nodes in heterobaric species, the bulk flow area was corrected by the following ratio:

(S31) $$\frac{\left(1-2w_{x,tot}/p_{bs}\right)a_{bs,out}}{a_{grid,bs-bse}}$$

The analogous corrections for homobaric species are identical except that the BSE width $w_{x,tot}$ is replaced by twice the width of the outermost column of tissue bands in these species, or $2\cdot r_{areole}/24$:

(S32) $$\frac{\left(r_{areole}/6p_{bs}\right)a_{bs,out}}{a_{grid,bs-bse}}, \text{ and}$$

(S33) $$\frac{\left(1-r_{areole}/6p_{bs}\right)a_{bs,out}}{a_{grid,bs-bse}}.$$

Computing volumes for each node

To compute volumes for each node, we consider each node to represent a three-dimensional annulus within the areole, bounded horizontally by cylinders and vertically by planes. For mesophyll nodes, the cylinders are chosen to bisect the lines connecting each adjacent node horizontally, and the planes are chosen to bisect the lines connecting each adjacent node vertically. For the upper and lower rows of nodes, which represent epidermis, we used measured upper and lower epidermis thicknesses (t_{eu} and t_{el}) to compute volumes. For BSE nodes, we used the measured BSE half-width ($w_{x,tot}/2$) to compute volumes. The calculations of hydraulic conductances presented in this study did not require computation of bundle sheath node volumes.

Example of spurious differences in BS and outside-BS hydraulic conductances arising from defining outside-xylem hydraulic conductance in terms of the average water potential of the entire symplast

In experimental studies, K_{ox} is defined in terms of leaf water potential, which equals the volume-weighted average water potential of the entire outside-xylem compartment (ψ_{ox}), provided that negligible water leaves the xylem during equilibration after excision. Thus, (S34) $K_{ox} = E/|\psi_{ox}|$.

In this study, we sought to partition K_{ox} into two serial pathways: the BS (with conductance K_b) and the outside-BS compartment (with conductance K_{ob}), such that

(S35) $$\frac{1}{K_{ox}} = \frac{1}{K_b} + \frac{1}{K_{ob}}.$$

where K_b is calculated from the water potential drawdown across the BS, or $\delta\psi_{bn}$ (the drawdown to grid nodes immediately adjacent to, or neighboring, BS nodes; hence the subscript *bn*):

(S36) $K_b = E/\delta\psi_{bn}$.

Combining S34-S36 gives K_{ob} as

(S37) $K_{ob} = E/(|\psi_{ox}| - \delta\psi_{bn})$.

The value of K_{ob} given by Eqn S37 is not uniquely defined by outside-BS water transport properties, however. Imagine two leaves that are identical in every respect except for the value of K_b; specifically, suppose leaf A has K_b twice as large as leaf B. The computed values of K_{ob} will differ for these two leaves, as demonstrated in the example shown in the table below, and the difference will depend on the volume fractions of the BS and outside-BS compartments. This spurious difference in calculated K_{ob} can be traced to the fact that K_{ob} is defined in terms of a water potential (ψ_{ox}) that includes some tissues (the BS) upstream of the tissues whose transport properties are meant to be characterised by K_{ob}. In the example below, this leads to a 63% difference in K_{ob} between the two leaves, even though they have identical outside-BS transport properties.

parameter	**leaf A**	**leaf B**
fraction of OX volume that is in BS (f_b)	0.1	
fraction of OX volume that is distal to BS (f_{ob})	0.9	
transpiration rate (E)	10	
water potential drawdown from outer edge of BS to site of average water potential of outside-BS tissues ($\delta\psi_{ob}$)	0.4	
BS hydraulic conductance (K_b)	50	25
water potential at outer edge of BS (ψ_{bn})	$-E/K_b = 0.2$	0.4
water potential of BS (ψ_b)	$\psi_{bn}/2 = -0.1$	-0.2
average water potential of outside-BS tissues (ψ_{ob})	$\psi_{bn} - \delta\psi_{ob} = -0.5$	-0.6
outside-xylem water potential (ψ_{ox})	$f_b \cdot \psi_b + f_{ob} \cdot \psi_{ob} = -0.46$	-0.56
outside-xylem hydraulic conductance (K_{ox})	$E/\lvert\psi_{ox}\rvert = 21.74$	17.86
outside-BS hydraulic conductance (K_{ob})	$1/(1/K_{ox} - 1/K_b) = 38.46$	62.54
% spurious difference in K_{ob}	63%	

We addressed this issue by defining K_{ox} in terms of the volume-weighted water potential drawdown to all tissues outside the BS ($\delta\psi_{ob}$; Eqn 10 in the main text).

Table S1. Flow areas per unit bulk area (γ) used to calculate bulk conductivities from intrinsic conductivities.

tissue	flow direction	symbol(s)	formula
apoplastic flow			
palisade mesophyll	vertical	$\gamma_{a,pv}$	$2p_a(1-p_p)t_{ap}/r_p$
palisade mesophyll	horizontal	$\gamma_{a,ph}$	$\frac{p_a(1-p_p)t_{ap}(2\pi r_p+2h_p)}{\pi r_p^2+2r_p(h_p-2r_p)}$
spongy mesophyll	both	$\gamma_{a,s}$	$2p_a(1-p_s)t_{as}/r_s$
epidermis	vertical	$\gamma_{a,ev}$	$4p_a t_{ae}/w_e$
epidermis	horizontal	$\gamma_{a,eh}$	$2p_a t_{ae}(h_e^{-1}+w_e^{-1})$
bundle sheath extensions	both	$\gamma_{a,x}$	$4p_a t_{ax}/w_x$
bundle sheath	-	$\gamma_{a,b}$	$4p_a t_{ab}/w_b$
transmembrane flow			
palisade mesophyll	vertical	$\gamma_{m,pv}$	$2(1-p_p)f_c$
palisade mesophyll	horizontal	$\gamma_{m,ph}$	$\frac{\pi(1-p_p)f_c h_p}{2h_p-(4-\pi)r_p}$
spongy mesophyll	both	$\gamma_{m,s}$	$2(1-p_s)f_c$
other	both	γ_m	1
gas phase flow			
palisade mesophyll	both	$\gamma_{g,p}$	p_p
spongy mesophyll	both	$\gamma_{g,s}$	p_s
other	both	γ_g	1

Table S2. Flow pathlengths per unit direct pathlength (β) used to calculate bulk conductivities from intrinsic conductivities.

tissue	flow direction	symbol(s)	formula
apoplastic flow			
palisade mesophyll	vertical	$\gamma_{\mathrm{a,pv}}$	$1+\left(\left(1-f_c\right)\pi-2\right)r_p/h_p$
palisade mesophyll	horizontal	$\gamma_{\mathrm{a,ph}}$	$\frac{1}{2}\left(1-f_c\right)\pi$
spongy mesophyll	both	$\gamma_{\mathrm{a,s}}$	$\frac{1}{2}\left(1-f_c\right)\pi$
other	any	γ_{a}	1
transmembrane flow			
all	any	γ_{m}	1
gas phase flow			
all	any	γ_{g}	1