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Where does Münch flow begin? Sucrose transport in the pre-phloem path

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Current conceptions of sucrose export largely neglect the effect of transpiration-induced water potential gradients within leaf mesophyll, even as the mix of convection and diffusion in the pre-phloem path remains uncertain. It is also generally held that the relative importance of convection and diffusion in the pre-phloem path is controlled by the ratio of their respective mass transfer coefficients. Here, we consider pre-phloem sucrose transport in the presence of adverse water potential gradients, finding that whether convection impedes or aids sucrose delivery to the phloem is independent of the permeability of the plasmodesmata to bulk flow, and depends only on assimilation rate, path-length, and the diffusivity. For most tissues subject to transpiration, convection through plasmodesmata pushes sugar away from the phloem.

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Introduction

In the leaves of symplastic passive loaders, plasmodesmal connections form a continuously connected symplastic space that links the cells of the photosynthetic mesophyll directly to the phloem [1,2]. In passive export, carbon assimilation creates a local increase in sucrose concentration that drives a diffusive flux of sucrose, and may also lead to a gradient in turgor that sets the cytosol in motion. If convection of the cytosol sweeps sucrose toward the phloem, Münch flow may then be said to begin in the mesophyll [3–5], as originally suggested by Münch himself [6]. Yet, current models of phloem loading focus on transport within the vein, and so treat the mesophyll/bundle sheath as a single cell, proximal to the xylem and phloem, with delivery replaced by a source term that implicitly assumes diffusive transport from the (neglected) distal mesophyll [7•,8]. Furthermore, during transpiration mesophyll tissues experience large water potential gradients [9] that may reverse the turgor gradient and so result in adverse (i.e. away from the phloem) convection. Daytime water potential gradients may therefore impede sucrose transport in two ways: first by reducing the phloem pressure difference from source leaves to stem and root sinks and so frustrating loading [10], and second, by creating adverse turgor gradients in the mesophyll that oppose sucrose delivery to the vascular bundle. Here, we focus on the latter problem, and make use of a simple model of pre-phloem transport to investigate the question of where Münch flow begins.

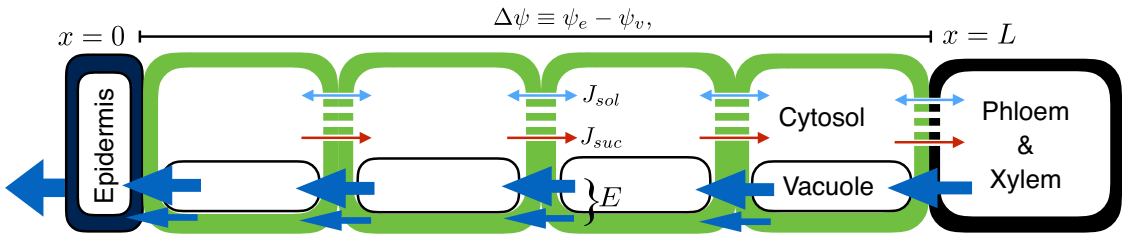
Modeling the pre-phloem pathway

We begin by considering the flux of sucrose within a linear file of mesophyll cells producing sucrose at a volumetric rate A (mol m^{-3} s^{-1}, the area normalized rate divided by the leaf thickness), and neglect the details of loading that occur in the vascular bundle itself (Box 1). Note that, given uncertainty about the relevant subcellular volume fractions, we express all concentrations and fluxes on a whole leaf basis [11•,12]. At one end, the cell file is bounded by epidermal cells symplastically isolated from the mesophyll [1], and the convective and diffusive fluxes of sucrose at this boundary must sum to zero. At the other end, to approximate the condition for passive loading, we impose that the sucrose concentration $c_{suc}(x)$ reaches a characteristic concentration for red oak phloem, c_p, of 500 mol m^{-3}, within the range shown for trees [13,14]. The global concentration of non-sucrose osmolytes, c_o, we set to 500 mol m^{-3}, and the water potential of the vein xylem we set to −1.1 MPa, such that the turgor of the palisade falls in the neighborhood of 1.4 MPa, as previously reported for red oak [15•]. That c_o is spatially invariant implies a non-modeled homeostatic process within each cell that counters the effects of convection.

As CO_2 diffuses into the leaf along its concentration gradient, water vapor diffuses outward, resulting in a flux of water from the stomata termed transpiration. Under steady-state conditions, a flux equivalent to transpiration moves from the veins to the stomata along multiple potential pathways: as liquid, through the cell wall apoplast, cell-to-cell across the walls and membranes of the mesophyll cells (mediated by aquaporins), or through the connected symplast of the mesophyll (mediated by plasmodesmata), and, as vapor, through the intercellular airspaces. Although cell pressure probe measurements

Box 1

Steady-state mesophyll sugar transport



Boundary conditions at $x=0$:

$$\frac{\partial J_{suc}}{\partial x}\Big|_{x=0} = 0$$

$$\psi(x=0) = \psi_e$$

Transpiration (across membranes, in apoplast)

Convection of cell solution (plasmodesmata)

Net flux of sugar (plasmodesmata)

$\Delta\psi$ water potential drop imposed by transpiration

Boundary conditions at $x=L$:

$$c_{suc}(x=L) = c_p$$

$$\psi(x=L) = \psi_v$$

Model development (main ideas)

Local water potential EQ: $\psi(x) = p(x) - RT(c_{suc}(x) + c_o) \longrightarrow \frac{\partial p}{\partial x} = -\frac{\Delta\psi}{L} + RT\frac{\partial c_{suc}}{\partial x}$

Dilute solution convection: $J_{sol} = Uc_{sol} \approx Uc_{water} \longrightarrow U = -\nu k_p \frac{\partial p}{\partial x}$

Convection-Diffusion: $\frac{\partial c_{suc}}{\partial t} = -\frac{\partial J_{suc}}{\partial x} + A = -\frac{\partial}{\partial x}(Uc_{suc}) + D\frac{\partial^2 c_{suc}}{\partial x^2} + A$

Governing equation

$$\frac{\partial c_{suc}}{\partial t} = 0 = -\frac{\nu k_p \Delta\psi}{L}\frac{\partial c_{suc}}{\partial x} + \nu k_p RT\left[\left(\frac{\partial c_{suc}}{\partial x}\right)^2 + c_{suc}\frac{\partial^2 c_{suc}}{\partial x^2}\right] + D\frac{\partial^2 c_{suc}}{\partial x^2} + A$$

Steady-state | Transpiration induced convection | Sugar induced convection | Diffusion | Sucrose production

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and swelling assays of individual cell permeabilities appear capable of explaining tissue level hydraulic conductivities in leaves without invoking large contributions from the cell wall apoplast [16], here we remain agnostic as to the whether the liquid flux occurs primarily across membranes or through the tangential cell walls. Our estimate of the water potential drop from veins to stomata that results from transpiration, though it accounts for vapor transport in the intercellular airspace, depends on experimental determinations of tissue hydraulic conductivity that does not discriminate between cross-membrane and apoplastic flows [9].

We do, however, assume that water potential gradients normal to the cell file are negligible relative to the gradient in water potential imposed by transpiration along the cell file. Thus, we regard the symplast and its tangent cell wall apoplast to be everywhere in 'local' equilibrium [17], an idea supported by modeling results that only a few percent of the transpiration flux evaporates directly from the spongy mesophyll cell surfaces far from the stomata [9,18]. We also ignore the effects of the convection of water through plasmodesmata on the overall water potential gradient through the cell file, which may be justified by noting that the plasmodesmatal flux is expected to be on the order of 1% of transpiration. We then describe the pressure (turgor) gradient driving convection through plasmodesmata as a function of the concentration gradients in sucrose and water potential (Box 1). Finally, we adopt a dilute solution approximation for the cytosol, based on the idea that the total concentration of osmolytes is small relative to the concentration of water molecules.

With the above framework, we avoid a detailed accounting of the symplastic water flux and arrive at a 'convection-diffusion' equation [19] for sucrose with a source and two boundary conditions that we solve numerically (Box 1). We parameterize the overall fluxes based on previous work on red oak leaves [9,15•], and adopt a range of plasmodesmal hydraulic permeabilities based on Comtet *et al.* [7•] and a range of effective diffusivities for cell-to-cell sucrose diffusion based on Liesche and Schulz [20] (Table 1). While both these transport parameters depend on plasmodesmal geometry, their precise relationship remains uncertain [7•]. We therefore independently vary the diffusivity of sucrose and the hydraulic conductivity of the plasmodesmata across different scenarios.

Pre-phloem transport in the context of negligible water potential gradients

To first approximate sucrose transport in a non-transpiring leaf, or in the palisade tissue of a transpiring leaf with no stomata on the upper epidermis, we set the water potential gradient $\Delta\psi/L$ to zero [9]. In this scenario, the first term of the governing equation vanishes (Box 2), and convection, like diffusion, is always toward the phloem. As a result, the convective and diffusive fluxes must both independently vanish at the epidermal boundary, and both fluxes grow linearly toward the phloem (Figure 1). In this case, whether convection or diffusion is more important is sensitive to the Peclet number P_e and so the ratio of k_p/D (Box 2). With the diffusivity set to the high value expected for well-coupled cells [20], the net sucrose flux is dominated by diffusion at low k_p, switching to convection-dominant at high k_p (Figure 1b–d). More significantly, in both cases the gradients in sucrose concentration and turgor required to drive sucrose to the phloem are vanishingly small, on the order of 1–10 mol m^{-3} or kPa (Figure 2a,b), and transport limitation in the pre-phloem path of the palisade, or in a non-transpiring leaf, appears very unlikely.

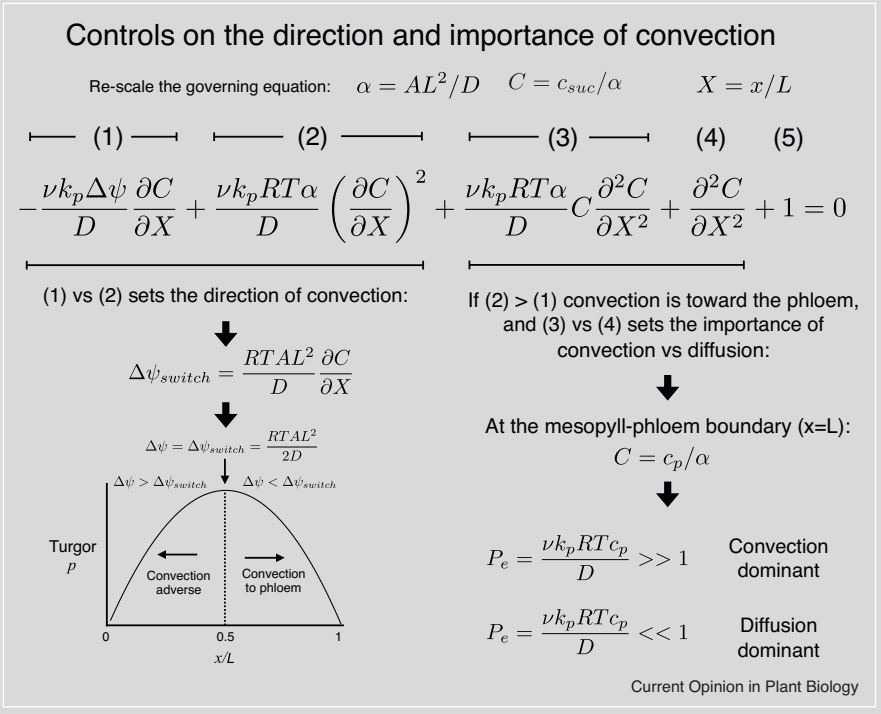
Of course, because the epidermis cannot be in steady-state a source of water, convection through the plasmodesmata requires an equal and opposite flux of water in the cross-membrane and apoplastic path to supply water to the mesophyll symplast. Like transpiration, this flux, though it enters the mesophyll rather than evaporating into the airspace near the stomata, imposes a water potential drop on the mesophyll. However, as the convective sap flux through the plasmodesmata (high *kp*, low or high *D* scenarios) is here on the order of 1% of the water flux across the spongy mesophyll during transpiration, the expected magnitude of the water potential drop required to supply this fluid is only ~6.6 kPa, a value beneath the magnitude of the threshold for adverse convection in the palisade of ~8 kPa (Box 2). Thus, the qualitative behavior is unaffected by the neglect of this small water potential drop, and the increase in peak sucrose concentration less than 1% if we instead account for it. Nevertheless, an additional small increase in the water potential drop due

Table 1

Physical quantities and constants used in the analyses

Quantity	Symbol	Value (25 °C)	Units	Citation
Assimilated carbon *c*, per area	A^c_{area}	20×10^{-6}	mol m^{-2} s^{-1}	[9]
Frac. of assim. *c* into sucrose	F_{suc}	0.77	–	[25]
Production of sucrose (area)	A_{area}	$F_{suc}A^c_{area}/12$	mol m^{-2} s^{-1}	
Production of sucrose (vol.)	A	$A_{area}\,(L_{spg}+L_{pal})^{-1}$	mol m^{-3} s^{-1}	
Mesophyll thickness, spongy	L_{spg}	100×10^{-6}	m	[9]
Mesophyll thickness, palisade	L_{pal}	160×10^{-6}	m	[9]
Cell length, spongy	l_c	25×10^{-6}	m	
Cell length, palisade	l_c	40×10^{-6}	m	
Gas constant	R	8.3145	m^3 Pa mol^{-1} K^{-1}	
Temperature	T	298.15	K	
Molar volume of water, liq.	υ	1.807×10^{-5}	m^3 mol^{-1}	
Permeability	L_{mp}	$5 \times 10^{-12} \rightarrow 1 \times 10^{-13}$	m Pa^{-1} s^{-1}	[7•]
Hydraulic conductivity	k_p	$L_{mp}\,l_c\,\upsilon^{-1}$	mol m^{-1} Pa^{-1} s^{-1}	
Cell coupling factor	CCF	$5 \times 10^{-7} \rightarrow 10^{-8}$	m s^{-1}	[20]
Diffusivity, sucrose	D	CCF l_c	m^2 s^{-1}	
Concentration of sucrose	c_{suc}	–	mol m^{-3}	
Conc. sucrose in phloem	c_p	500	mol m^{-3}	[13]
Conc. of water	c_{water}	$1/\upsilon$	mol m^{-3}	
Conc., other solutes	c_o	500	mol m^{-3}	[15•]
Water potential, vein	ψ_L	-1.1×10^6	Pa	[15•]
Water potential	ψ	–	Pa	
Epidermal ψ – vein ψ	$\Delta\psi$	$-0.66 \times 10^6 \rightarrow 0$	Pa	[9]
Turgor pressure	$_p$	–	Pa	
Convective velocity	U	–	m s^{-1}	
Total convective flux	J_{sol}	–	mol m^{-2} s^{-1}	
Total sucrose flux	J_{suc}	–	mol m^{-2} s^{-1}	

Box 2



to cuticular transpiration could be enough to tip the palisade into adverse convection (Box 2). However, because the total water potential drop remains small, the impact on the magnitude of the turgor and sucrose concentrations of the cell file would be negligible.

What then is the situation when the water potential drop is not small and convection is adverse? This is the regime that characterizes the spongy mesophyll during transpiration, to which we now turn.

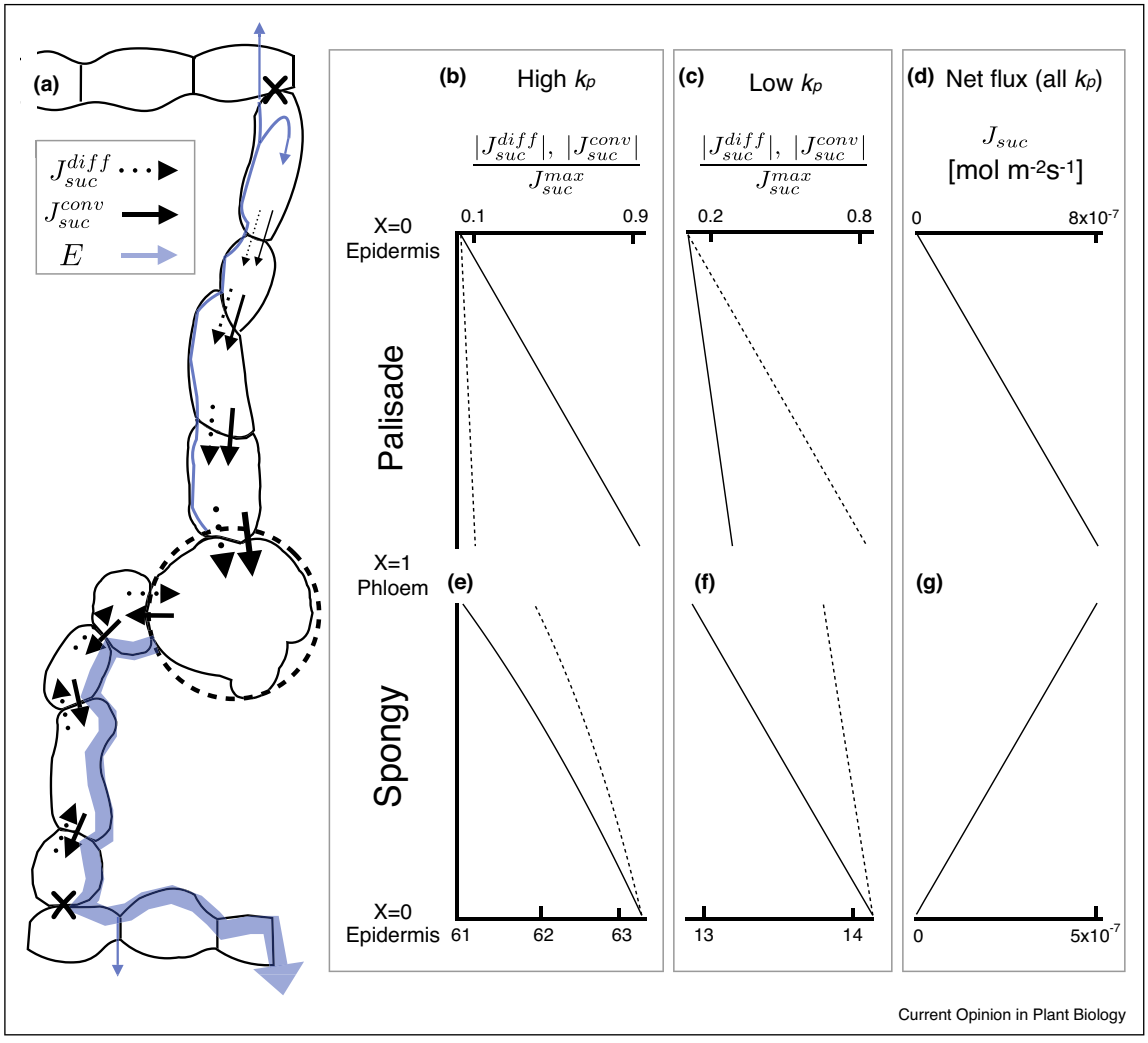
Controls on the direction of convection given large water potential gradients

For a sun-exposed red oak leaf at the top of a canopy, the expected water potential drop imposed on the spongy mesophyll by transpiration (−0.66 MPa) results in adverse convection over the whole parametric range (Figure 1e,f). The convective and diffusive fluxes of sucrose cancel each other at the epidermal boundary, yet toward the phloem the net flux grows linearly as their difference, rather than as their sum, as in the palisade (or the case of minimal transpiration). Thus, in the spongy mesophyll of a transpiring leaf both the diffusive and convective sucrose flux can be an order of magnitude larger than the net flux. The diffusive and convective fluxes are also nearly constant along the length of the cell file; it takes only a small but increasing difference in the opposing fluxes to create a net transport of sucrose that balances the sucrose production rate (Figure 1g).

For a higher value of k_p, more sucrose is swept back toward the epidermis, raising the concentration to drive a larger diffusive flux (Figure 2c). This increase in sucrose concentration also increases the turgor pressure at the epidermal boundary, but the effect saturates and the turgor there approaches, but can never exceed, the value at the phloem end of the cell file (Figure 2d). Adverse convection cannot drive the sucrose concentration at the epidermal end high enough to invert the turgor gradient (i.e. pushing toward the phloem), simply because it is convective flow toward the epidermis that creates the high sucrose concentration at that boundary in the first place.

Other factors can, despite the existence of an adverse water potential gradient, lead to the development of a turgor gradient toward the phloem. A higher sucrose production rate A, a longer cell file L (due to an increase in cell number), or lower diffusivity D all favor a local accumulation of sucrose that can overcome a transpiration-induced potential drop $\Delta\psi$, and so switch convection through the plasmodesmata from toward the epidermis to toward the phloem (Box 2). A, L, and D define a particular value $\Delta\psi_{switch}$ at which the cell file is poised between adverse and phloem-ward convection, with a shallow but

Figure 1

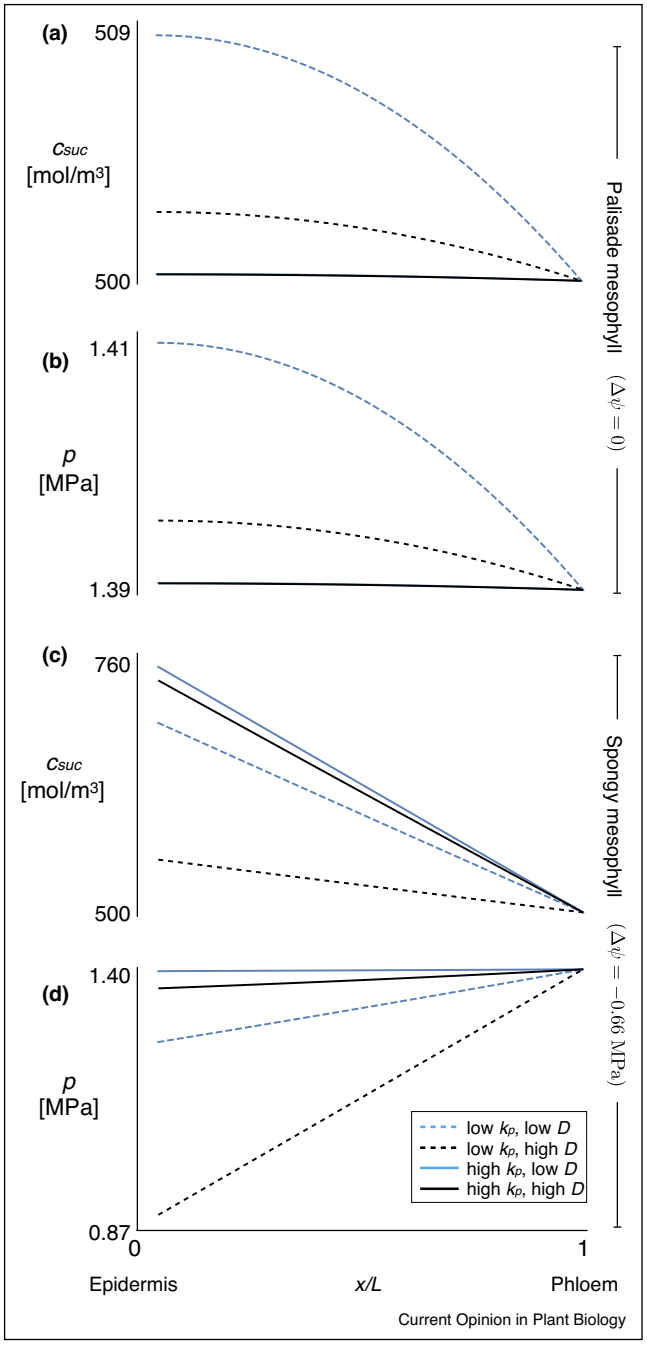


Magnitudes of the convective, diffusive, and net mesophyll sucrose fluxes. **(a)** Diffusive (dotted arrow) and convective (solid arrow) fluxes of sucrose from mesophyll to minor vein; X marks the lack of symplastic connectivity, a dashed circle indicates the boundary where $c_{suc}(x) = c_p$, and the cross-membrane and apoplastic liquid and vapor water fluxes are in blue. In the spongy, this water flux is large due to transpiration, and the turgor gradient and convective flow oppose sucrose diffusion. In the palisade, small cuticular and circulatory fluxes of water result in a water potential drop that is, to a first approximation, negligible. Both convection and diffusion are toward the vein and their relative importance switches between high **(b)** and low **(c)** values of k_p. In the spongy **(e,f)**, convection is away from the vein, and the net flux emerges from a small difference between the two component fluxes: note that the magnitudes of diffusion and convection are both many times larger than the net flux, and both are nearly constant through the tissue. **(d,g)** The total net flux in each tissue results from the linear accumulation of assimilate due to constant A, with the maximum net fluxes occurring at the vein boundaries used to normalize the component fluxes in b,c,e,f.

inverse parabolic turgor gradient. For higher rates of transpiration, and so larger $\Delta\psi$, the system evolves toward a linear turgor gradient toward the epidermis (Figure 2c) and adverse convection over the whole cell file (Figure 1e, f). For reductions in $\Delta\psi$, turgor-driven convection facilitates diffusion as discussed for the $\Delta\psi = 0$ case (Figures 2d and 1b,c).

When mesophyll cells are well-coupled by plasmodesmata [20] and within four to five cells of the phloem [4], diffusive transport of sucrose is efficient and the magnitude of $\Delta\psi_{switch}$ is expected to be two orders of magnitude below that of the $\Delta\psi$ imposed by transpiration (for oak, −8 kPa versus −0.66 MPa). We therefore expect that mesophyll tissues located between the vasculature and a transpiring epidermis will, in general, be in a regime of significant adverse convection. In this case high k_p and low D increase the sucrose concentration gradient and lead to nearly uniform and high mesophyll turgor. Conversely, low k_p and high D lead to small sucrose concentration gradients, and large adverse turgor gradients (Figure 1). Note that the model behavior reported

Figure 2



Sucrose concentration c_{suc} and turgor p in the palisade **(a,b)** and spongy **(c,d)** mesophyll of a transpiring, hypostomatous leaf: solid = high, dashed = low plasmodesmal hydraulic conductivity k_p; black = high, blue = low diffusivity for sucrose through plasmodesmata D. In the palisade, convection and diffusion combine such that concentration (a) and turgor (b) gradients all but vanish. In the spongy, as k_p increases and or D decreases, more sucrose is swept toward the epidermis until concentrations (c) rise high enough for diffusion to drive a net flux toward the phloem. As a result of an increasing sucrose gradient, the turgor (d) gradient driving adverse convection diminishes, resulting in a saturating effect of higher k_p/D on both concentration and turgor.

here is independent of c_o and the absolute water potentials, and so absolute turgor values, specified by our parameterization.

Derived quantities calculated from the model appear plausible as well. Convective velocities range from around $\sim$50 nm s^{-1} for the low D, high k_p scenario, to $\sim$4 nm s^{-1} for high D low k_p. As a point of reference, the slab velocity for transpiration is here 100 nm s^{-1}. While these velocities would increase when scaled from a leaf to a symplast area basis, they seem likely to remain sufficiently small relative to cytoplasmic streaming velocities (expected magnitudes on the order of μm s^{-1} [21]), such that *within an individual cell* neither convection nor transpiration biases metabolite transport to an important extent. Finally, the cell-to-cell turgor differences predicted here are below the threshold for plasmodesmatal gating (reported for *Nicotiana tabacum*) of 0.2 MPa [22].

How might constraints arising from pre-phloem transport affect leaf structure?

A central feature that emerges from our investigation is the competence of diffusion in both the presence and absence of adverse convection. Even when transpiration rates are high, the resulting water potential gradients within the leaf do not prevent the leaf from transporting sucrose to the phloem. We note, however, this conclusion depends on similar diffusivities for fluorescein and sucrose through plasmodesmata, an assumption supported by their similar size and diffusivities in other media [20]. We also have little idea of, and so neglect, the costs of maintaining local homeostasis in important metabolic molecule concentrations (i.e. c_o) in a well-connected symplastic space exposed to convective flows.

Given the persistence of adverse convection during transpiration over the explored parameter space, we fully expect that the behaviors predicted here generalize beyond red oak. While larger mesophyll air fractions may lead to more temperature driven vapor transport within a leaf, mitigating in part the water potential gradients that arise due to liquid phase transport of the transpiration flux, the effect is unlikely to prevent adverse water potential gradients. For example, temperature driven vapor transport accounts for less than 15% of the total flux in a leaf with a 30% volumetric air fraction in the spongy mesophyll [9]. With respect to other loading types, although we have focused on a passive loading species to illustrate the model, we also expect our sketch of pre-phloem transport should translate to active loaders as well. While for active loaders the sucrose concentration near the phloem would be lower than the value of c_p imposed on the vascular bundle boundary here, that parameter only affects the magnitude of the absolute concentrations achieved in the mesophyll, and not the general behavior of the gradients and fluxes. Coupling a model of pre-phloem transport to future loading models

will allow accounting for the effects of both phloem-ward and adverse symplastic convection on the concentrations and flows occurring in the loading zone.

From an ecophysiological perspective, the expected efficiency of pre-phloem transport within the parameter space occupied by leaves is not surprising given the importance of moving sucrose from the sites of assimilation to heterotrophic sinks. Indeed, to understand how the demands of pre-phloem transport may constrain leaf structure, we need to consider what happens on the boundaries of the parameter space. An obvious candidate is cell number, or how far a mesophyll cell can be from the phloem and still export sucrose efficiently. In the spongy mesophyll at least, transpiration-induced water potential gradients are expected to grow linearly with cell number, requiring ever larger sucrose concentrations at the epidermal boundary to reduce adverse turgor gradients and thus allow diffusion to beat convection. Such effects could, for example, contribute to the association between high light, thick leaves and amphistomy [23,24]. This is because, assuming symmetric leaf properties above and below the veins, redistributing stomata from the lower to the upper surface produces an equivalent flux (neglecting boundary layer effects) that conserves bulk leaf transpiring water potential, but halves the magnitude of the gradients by splitting the flux between two transpiring surfaces. That said, with respect to transpiration-induced constraints on export, our analysis suggests that leaf-to-sink xylem gradients in water potential are a more likely source of constraints on daytime phloem loading than are the gradients that develop within a leaf between vasculature and stomata.

Acknowledgements

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