

Loading regulation prevents phloem failure during drought and widens the range of phloem and stomatal traits

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June 14, 2021

Abstract

Plant carbon transport is controlled by a multitude of parameters both internal and external to the sugar transporting phloem tissue. Sucrose transporter kinetics, conduit hydraulic resistance, and xylem water stress are all hypothesized to impact the amount of carbon delivered to sink tissues. However, the most important traits determining carbon export under drought are not well understood, especially for species with active molecular regulation of sucrose transport. This in turn limits our ability to assess species' resistances to phloem dysfunction under drought. Here, we use an integrated xylem-phloem-stomatal model to calculate leaf water potential from soil dryness, which is then used to determine gas exchange and phloem pressure gradients. We quantitatively compare the impacts of phloem loading kinetics, including feedbacks between loading and phloem pressure, phloem conduit resistances, and stomatal responses to water stress, on the total carbon export to sinks during drought. Regulating sucrose transporter kinetics which downregulates loading at high phloem pressures prevented runaway viscosity in the phloem sap and was the most important determinant of export rates under drought. In contrast to previous models, we found this feedback mechanism decoupled stomatal traits from phloem export efficiency during drought and increased the operational range of phloem hydraulic resistances.

Introduction

The phloem tissue is the “enigmatic central banker” appropriating and transporting carbon from the photosynthesizing sources to the carbon-consuming sinks (Ryan and Asao 2014). For the plant to “cash-out” the carbon-rich phloem sap for growth, respiration, or storage, the phloem must maintain a pressure gradient from source to sink to drive bulk flow (Münch 1930, van Bel 2003). A wide range of xylem and stomatal traits have been linked to maintaining plant hydraulic function under water stress and adapting plants to dry environments (Bartlett et al. 2016, Meinzer et al. 2009). However, the traits that confer phloem drought tolerance by maintaining pressure gradients for carbon transport under water stress are not well understood, due to the technical difficulty of measuring phloem transport *in vivo* (Savage et al. 2016, Jensen et al. 2016). Therefore, modeling approaches have been critical to assess the impacts of phloem anatomical traits and the hydraulic status of the plant on phloem transport (e.g., Jensen et al. 2012, Thompson and Holbrook 2003a,b). Coupled phloem-xylem transport models suggest that phloem transport is highly vulnerable to drought stress (Daudet et al. 2002, Hölttä et al. 2006, 2009, Lacomte and Minchin 2008) and that conservative stomatal behavior may reduce these impacts (Huang et al. 2018). However, although many of these past coupled models make phloem loading concentration dependent, they ignore Michaelis-Menten formalism for protein assisted active loading of sugars into source tissue. This is surprising considering work that shows up to 42% of species surveyed utilize an active sugar loading mechanism that would require membrane bound proteins (Rennie and Turgeon 2009); this loading mechanism includes economically important crops such as celery, tobacco, spinach, tomato, cotton, sunflower, and grapevine (Davies et al. 1999, Muller et al. 2014, Rennie and Turgeon 2009). The molecular mechanisms regulating active transport could provide the phloem with an additional level of control over transport (eg., Xu et al. 2018, Xu et al. 2020, Bush 2020), and the

alteration of this control has been hypothesized to be important for genetic engineering of crops to increase yield (Braun et al. 2014). However, the mechanistic hydraulic consequences for phloem transport under stress are largely unknown. Thus, we conducted the first study to our knowledge evaluating the impacts of these regulatory mechanisms, and the interactive effects of transporter kinetics, phloem anatomy, and stomatal behavior on sugar translocation and export under drought.

In species with active phloem loading, the phloem conduit plasma membranes exhibit molecular regulatory mechanisms that change the capacity to load sugars for transport. In these species, membrane transporter proteins lining the phloem conduit membranes (sieve tubes) load sugars from the surrounding extracellular space (apoplasm) in leaf tissue (Rennie and Turgeon 2009). This decouples loading rates from the concentration gradient between the mesophyll and phloem (Lalonde et al. 2004, De Schepper et al. 2013, Milne et al. 2018). In contrast, passive loading requires there to be a favorable osmotic and pressure gradient between sugar producing mesophyll and sieve tube cells for phloem loading to occur (Rockwell et al. 2018, Schulz 2015). Active loading, meanwhile, does not depend on this gradient and instead relies on the enzyme kinetics of sucrose loading proteins such as SUCs or SUTs (Lalonde et al. 2004); the rate of loading is highly dependent on the loaders maximum loading rate or affinity for sucrose (V_{max} or K_m values in Michaelis-Menten formalism). Phloem in the unloading zone can use a similar process to actively export sugars to the sink tissue (Hackel et al. 2006, Gould et al. 2013, Santi et al. 2013, Zhang et al. 2004, 2006) which is hypothesized to extend along the entire transport pathway (Thompson 2006). This is evidenced by sucrose transporter localization in both passive (Payyavula et al. 2011) and active (Wiese et al. 2000) loaders throughout stem phloem. Drought, heat, and light intensity impact the transcript abundance and post-translational modification of sucrose transporters in phloem tissue (Durand et al. 2018, Medici et al. 2014, Xu et al. 2018, 2020). This may impact sucrose loading rates in phloem leading to downstream impacts on sugar export to sinks (Ainsworth and Bush 2011, Bush 2020). However, it remains unclear by what mechanism maximum loading and unloading rates across species and changes in sucrose transporter activity impacts the ability of phloem conduits to move carbon from source to sink, especially during drought.

Sucrose loading and unloading are critical to phloem drought responses. Drought stress impacts phloem transport by making it more difficult for water to enter the phloem. As water potential declines in the leaf xylem, the phloem must compensate by increasing sugar concentrations in the loading zone, to reduce osmotic potentials and attract water from the neighboring xylem (Konrad et al. 2019). This influx of water from the xylem generates the high turgor pressure in the loading zone that powers source-to-sink carbon transport. However, this presents a conundrum, since higher sucrose concentrations increase the viscosity of the phloem sap and, thus, the hydraulic resistance of the phloem (Sevanto 2014, 2018, Salmon et al. 2019). In well-watered conditions, sugar concentrations have been demonstrated to be optimal for efficient flow in many species (Jensen et al. 2013). However, under drought, the sugar concentrations needed to draw water from the xylem can increase viscosity to the point that the pressure gradient is unable to overcome phloem resistance, generating a positive feedback between loading and resistance that leads to transport failure (i.e., ‘run-away viscosity’) (Hölttä et al. 2009, Nikinmaa et al. 2013, Sevanto et al. 2014). The excessive pressure generated in the loading zone during this viscosity ramp up may also conceivably cause leakage of sucrose back out into the apoplasm, lowering overall transport efficiency, but this to our knowledge has yet to be tested. Reducing phloem turgor by experimentally imposing osmotic stress has been shown to increase loading rates (Smith and Milburn 1980), indicating that active-loading species can regulate loading rates in response to environmental conditions, sensed via the phloem water status. However, the impacts of phloem loading regulation to the ‘run-away viscosity’ problem in the context of drought is unknown.

Since a run-away viscosity event is due to excessive resistance occurring along the transport pathway, the structural resistance of the sieve tube may also impact this type of transport failure. However, a recent meta-analysis shows that the sieve tube anatomical resistance of a species bears little relation to where it is located across biomes (Liesche et al. 2017). This suggests that the availability of water to the plant shows little relation to its phloem anatomical properties. This is surprising as increased phloem structural resistance should make the plant more vulnerable to run-away viscosity, especially during drought. Structural aspects of the phloem that contribute to resistance may be in the form of its cell diameter and the porosity of their end

walls (sieve plates) (e.g., Mullendore et al. 2010, Stanfield et al. 2019). In addition, the scaling of the conduits from source-sink may also play a role to minimize resistance and maintain adequate pressure gradients in both leaves and stems (Carvalho et al. 2017, Savage et al. 2017); this scaling increases in magnitude as pathway resistance per unit length lowers in taller trees to maintain flow at great heights (Clerx et al. 2020). Thus, although there is evidence to suggest resistance scales to maintain carbon transport within a plant, linking this parameter across species and environmental conditions (e.g., drought) does not seem clear.

Previous models suggest that plants close stomata under drought partly to mitigate impacts on phloem transport. More isohydric stomatal behavior (i.e., stomatal closure under less severe water stress) prevented xylem water potentials from declining to the point that passive loading was osmotically unfavorable (Huang et al. 2018). When loading rates were assumed to equal photosynthetic rates, more isohydric stomatal behavior prevented phloem concentrations from reaching thresholds for runaway viscosity (Nikinmaa et al. 2013). These findings have suggested that phloem transport strongly constrains gas exchange during drought. However, previous work has not addressed the potential for feedbacks between loading, plant water status, and architecture to prevent runaway viscosity and decouple stomatal behavior from phloem transport in active-loading species. Elucidating how stomatal behavior, phloem loading kinetics, and phloem structure interact to determine sucrose export to sinks under drought is crucial to identify the key traits conferring stress tolerance to carbon transport. These traits could then be applied to assess species' vulnerabilities to carbon starvation under the drier conditions expected under climate change or develop crop cultivars with more drought tolerant carbon transport systems.

Previous models have addressed how aspects of water deficit (Hölttä et al. 2006, 2009, Zhou et al. 2019), phloem architecture (Hölttä 2009, Jensen et al. 2012, Stanfield et al. 2019), loading and unloading kinetics (De Schepper and Steppe 2010, Hölttä et al. 2006, Goeschl and Han 2020), and stomatal behavior (Nikinmaa et al. 2013, Huang et al. 2018) impact phloem transport. However, they have not synthesized these components to quantify the relative importance of these variables to sucrose export under drought. The overall goal of this model is to determine how phloem anatomy, transporter kinetics and stomatal control traits influence sugar transport under a variety of plant water statuses. Specifically, this study addresses the questions: 1) what are the soil water potential thresholds that limit sugar export from the phloem in apoplastic loading and unloading species? 2) how are these thresholds impacted by changes in phloem loading and unloading kinetics, phloem hydraulic resistance and sucrose leaking back out of the loading zone? and 3) how does stomatal behavior impact sugar export under water stress? We address these questions by integrating a stomatal-hydraulic model (modified from Bartlett et al. 2019) that calculates leaf water potentials and gas exchange rates from environmental and hydraulic trait inputs. We then combine this with a simple phloem transport model (similar to the axial transport model of DeSchepper and Steppe 2010) that calculates phloem pressure gradients and flow rates from leaf sucrose concentrations and water potentials, using the Michaelis-Menten enzyme formalism to represent active loading and unloading (Fig. 1).

Materials and Methods

Model overview

Conceptually, the model can be divided into three components: plant water status, gas exchange and phloem transport (Fig. 1). The plant water status component calculates the leaf mesophyll and xylem water potentials (Ψ_m and Ψ_x) from an input soil water potential (Ψ_s) and gas exchange and hydraulics. The gas exchange component calculates photosynthesis from the stomatal aperture, which is determined from the leaf water status (Ψ_m). The phloem transport component calculates a source – sink phloem concentration and pressure gradient generated from the Michaelis-Menten kinetics of loading/unloading sucrose and the water stress experienced by the phloem (Ψ_x or Ψ_s). Here we assume the plant is an apoplastic loader and unloader.

Plant water status and gas exchange

We separated the plant into two hydraulic elements, the mesophyll of a single leaf and the root-to-leaf xylem network, to capture the protective effect of vulnerability segmentation (Tyree and Ewers 1991). The leaf accounts for at least 30% of whole-plant hydraulic resistance, with the mesophyll tissue accounting for

about half of this resistance (Sack and Holbrook 2006, Scoffoni et al. 2011). This generates a strong water potential gradient across the mesophyll that buffers the xylem and phloem against water stress. We adapted the water balance equations from Sperry et al. (1998) to calculate the mesophyll and xylem water volume at each timestep:

$$\frac{dW_m}{dt} = \int_{\Psi_m}^{\Psi_x} K_m d\Psi - g_s VPD \quad \text{Eqn 1a}$$

$$\frac{dW_x}{dt} = K_x (\Psi_s - \Psi_x) - \int_{\Psi_m}^{\Psi_x} K_m d\Psi = 0 \quad \text{Eqn 1b}$$

where W is the water volume, Ψ is the water potential, and K is the hydraulic conductance of the mesophyll (subscript m) and xylem (subscript x). VPD and Ψ_s are the environmental parameters, the vapor pressure deficit and soil water potential, respectively; g_s is the stomatal conductance, and LA is the area of a single leaf (see Table 1 for constant parameter values and Table 2 for the parameters varied across simulations). Here we assumed the water volume in the mesophyll changes over time, while the xylem is at steady-state (i.e., $\frac{dW_x}{dt} = 0$).

The mesophyll water volume is converted to a relative water content by dividing by the maximum water volume ($RWC_m = \frac{W_m}{V_{sat, m}}$), and then to a water potential through the pressure-volume relationships:

$$\Psi_m = \begin{cases} \frac{\pi_m}{RWC_m} & \Psi_m > \pi_{tlp} \\ \frac{\pi_o (1-a_f)}{RWC_m - a_f} - \pi_o - \epsilon \left(1 - \frac{1-a_f}{RWC_m - a_f}\right) & \Psi_m \leq \pi_{tlp} \end{cases} \quad ; \text{Eqn 2}$$

where π_o , π_{tlp} , a_f , and ϵ are the leaf pressure-volume curve parameters of osmotic potential at full hydration turgor loss point, apoplastic fraction, and cell wall modulus of elasticity, respectively (Bartlett *et al.* 2012). We considered the effects of mesophyll sugar content on water relations to be outside the scope of this study, and, thus, we assumed here that π_o was constant.

Water flow through the mesophyll was determined by integrating the mesophyll vulnerability curve:

$$K_m = \frac{K_{max, m}}{1 + e^{-\alpha(\Psi_m - \Psi_{50, m})}} \quad \text{Eqn 3}$$

where $K_{max, m}$ is the maximum hydraulic conductance of the mesophyll, normalized by leaf area, α is a shape parameter, and $\Psi_{50, m}$ is the mesophyll water potential at which 50% of conductance is lost. To simplify these calculations, we used a sufficiently negative xylem Ψ_{50} value (-2 MPa) to assume K_x was constant and equal to the maximum xylem conductance ($K_{max, x}$) over the range of xylem water potentials in these simulations.

We calculated g_s from the assumption that mesophyll water stress induces stomatal closure:

$$g_s = \frac{g_{max}}{1 + e^{-\alpha g_s (\Psi_m - g_s \Psi_{50})}} \quad \text{Eqn 4}$$

where g_{max} is the maximum stomatal conductance, αg_s is the shape parameter for this relationship, and $g_s \Psi_{50}$ is the mesophyll water potential inducing 50% stomatal closure. Photosynthesis (A_{net}) was then calculated from g_s based upon an extrapolation of the original Farquhar equations (Farquhar et al. 1980). We assumed that light was constant and non-limiting for photosynthesis over these simulations.

At the beginning of each timestep, Ψ_m was calculated from the change in mesophyll volume over the previous timestep (Eqns 1a, 2). Mesophyll water flow was then calculated by integrating the mesophyll vulnerability curve over water potential, bounded by the new Ψ_m and the Ψ_x from the previous timestep (Eqns 1, 3). Stomatal conductance (g_s) was then calculated from the new Ψ_m (Eqn 4), and Ψ_x was updated from the new mesophyll water flow

$$\Psi_{x, t} = \Psi_s - \frac{\int_{\Psi_{m, t}}^{\Psi_{x, t-1}} K_{m, t} d\Psi}{K_{max, x}} \quad \text{Eqn 5}$$

where t indicates the current timestep and used to determine the new xylem flow ($K_{max, x} (\Psi_s - \Psi_x)$) (Eqn 1b). The new mesophyll flow and g_s values were then supplied to Eqn 1a to update the mesophyll volume for the next timestep. We ran the model at a 1s timestep over 12hr simulations to achieve steady-state solutions, which were reported as the model results.

Phloem Transport

The mass of sucrose in the mesophyll (S_m) was increased by photosynthesis and reduced by loading into the phloem companion cells via sucrose transporters:

$$\frac{dS_m}{dt} = A_{\text{net}}LA - L_{\text{sucL}}LA \text{ Eqn 6}$$

where L_{sucL} is the loading rate per unit leaf area. To simplify the model, we assumed all the sugar produced by photosynthesis is exported into the apoplasmic space surrounding the sieve element/companion cell complexes of the loading zone, and thus available for phloem loading. For loading (subscript $i = L$) or unloading (subscript $i = U$), $L_{\text{suc},i}$ was calculated from the Michaelis-Menten formalism for enzyme kinetics:

$$L_{\text{suc},i} = V_{\text{max},i} \frac{C}{M_S + C} \text{ Eqn 7a}$$

$$C_m = \frac{S_m}{W_m} \text{ Eqn 7b}$$

where $V_{\text{max},i}$ is the maximum loading or unloading rate of the sucrose transporters, normalized by leaf area, and M_S is a shape parameter capturing transporter affinity for sucrose. C is either C_m , the sucrose concentration in the mesophyll outside the loading zone, or C_{pU} , the sucrose concentration in the unloading phloem zone. W_m is the mesophyll water volume (Eqn 1a). We assumed the transport phloem is impermeable, and water and sucrose are only exchanged in the loading and unloading zones.

Loading increases the mass of sucrose in the phloem loading zone ($S_{p,L}$), which is transported to the unloading zone or leaked back into the mesophyll through diffusion

$$\frac{dS_{p,L}}{dt} = L_{\text{SUCL}}LA - E_s - S_l \text{ Eqn 8}$$

where E_s is the mass flow rate of sucrose transport (g s^{-1}) and S_l is the mass flow rate of leakage. E_s is calculated from the volumetric flow rate of the phloem sap (F_p ; m^3s^{-1}) and sucrose concentration in the loading zone ($C_{p,L}$; mol m^{-3}):

$$E_s = F_p C_{p,L} \text{ mm Eqn 9a}$$

$$C_{p,L} = \frac{S_{p,L}}{W_p} \text{ Eqn 9b}$$

where mm is the molar mass of sucrose and W_p is the maximum water volume in the loading zone. Thompson and Holbrook (2003a) found that accounting for changes in phloem volume did not substantially impact the flux of sucrose through the transport pipeline. Thus, we made the simplifying assumption that W_p was constant at the maximum phloem cell volume, so that $C_{p,L}$ only varied with the mass of sucrose. We calculated W_p from the mean sieve element area, measured for a *Populus tremula x alba* leaf (Carvalho et al. 2017), and the total vein length in a grapevine leaf, as a representative apoplasmic loader (Pagano et al. 2016), assuming all leaf phloem is included in the loading zone. We calculated leakage as:

$$S_l = L_{\text{leak}} LA(C_{p,L} - C_m) \text{ Eqn 10}$$

where L_{leak} is the membrane permeability of the loading zone to sucrose diffusion (m s^{-1}), and the concentration gradient is the driving force for sucrose leakage.

The sucrose concentration determines the osmotic potential (π_i) in the loading (L) or unloading zone (U)

$$\pi_i = -RcTC_{p,i} \text{ Eqn 11}$$

where Rc is the gas constant and T is the temperature within the phloem loading area. Following Thompson and Holbrook (2003b), we made the simplifying assumption that the water potential of the phloem loading zone equilibrates with the xylem water potential (Ψ_x) when the phloem is at steady state. Further, we estimate that the anatomical dimensions, osmotic strength, and enhanced permeability due to aquaporins (Muries et al. 2013) of our loading zone has an RF value $\gg 1$ (see Thompson and Holbrook 2003b, Eqn 22), which equates to phloem in water potential equilibrium with the xylem. The turgor pressure in the loading or unloading zone (P_i) was then determined from the water and osmotic potentials:

$$P_i = \Psi_x - \pi_i \text{ Eqn 12}$$

noting that for determining the phloem pressure in the unloading zone, Ψ_s is used. The volumetric phloem flow rate F_p ($\text{m}^3 \text{s}^{-1}$) was calculated from the pressure difference between the loading (P_L) and unloading (P_U) zones and the hydraulic resistance of the phloem (R_{conduit})

$$F_p = \frac{P_L - P_U}{R_{\text{conduit}}} \text{ Eqn 13}$$

which increased with the phloem sucrose concentration due to viscosity (v) effects (see Appendix A), and integrated into a resistance formula:

$$R_{\text{conduit}} = R_p * v \text{ Eqn 14}$$

where R_p is the resistance coefficient of the phloem conduit (see Table 2).

The velocity of sap flow (V_s) was calculated by normalizing volumetric flow by conduit area

$$V_s = \frac{F_p}{\pi r^2} \text{ Eqn 15}$$

where r is the radius of the conduit.

The same processes then take place when the sucrose reaches the unloading zone, where unloading is determined from:

$$\frac{dS_U}{dt} = E_s - L_{\text{SUCU}} \text{ LA Eqn 16}$$

We made the simplifying assumptions that the source and sink area (LA) and the phloem volume in the loading and unloading zones (W_p) are equal.

Finally, we quantified sugar export (S_{tot}) as the cumulative mass of sucrose unloaded over the simulation, as

$$S_{\text{tot}} = LA \int_0^T L_{\text{SUCU}}(t) dt \text{ Eqn 17}$$

Model parameterizations to test hypotheses

We tested our hypotheses for the impacts of phloem hydraulic resistance and membrane transporter kinetics on phloem transport under water stress by varying the soil water potential (Ψ_s), phloem resistance to pure water (R_p), maximum loading and unloading rates ($V_{\text{max}L}$ and $V_{\text{max}U}$), membrane permeability to sucrose leakage (L_{leak}), and relationship between $V_{\text{max}L}$ and the loading zone turgor across simulations (see Table 2 for parameter values). First, we selected $5R_p$ values that generated phloem pressures (P_L) from across the observed range (i.e., 0.6 – 2.4 MPa) (Turgeon 2010) for well-watered conditions (here, $\Psi_s = 0$ MPa). Second, we varied $V_{\text{max}L}$ in simulations where unloading did not limit phloem transport (i.e., all sucrose that reached the unloading zone was exported in the same timestep; $V_{\text{max}U} \gg V_{\text{max}L}$), and simulations where unloading was limiting (i.e., sucrose can build up over time in the unloading zone; $V_{\text{max}U} [?] V_{\text{max}L}$). Phloem pressure in the unloading zone was ~ 0 MPa in the “unloading unlimited” and $\gg 0$ MPa in the “unloading limited” simulations. Third, we tested L_{leak} values measured for carrot callus tissue as an approximation for sucrose leakage out of loading specific phloem (Edelman and Schooler 1971). Fourth, we imposed turgor limitations on loading by setting $V_{\text{max}L} = 0$ when $P_L = 2.4$ MPa, which emerged as a consistent threshold for runaway viscosity (see Results). For all these simulations, we characterized responses to water stress by varying Ψ_s from -0.001 to -1 MPa at 0.1 MPa intervals. We compared phloem pressure (P_L and P_U), velocity (V_s), viscosity (v), and total sucrose export (S_{tot}) across these simulations.

To address the impacts of stomatal behavior on phloem transport, we varied the maximum stomatal conductance (g_{max}) and mesophyll water potential threshold for 50% stomatal closure ($g_s \Psi_{50}$) values. During these simulations, we varied the range of phloem resistance coefficients, and loading rates (see Table 2) to determine the interactions between adjusting these parameters.

Meta-analysis on phloem resistance and leaf water potential

Finally, we performed a meta-analysis to address if total phloem resistance values correlated with plant water status and hydraulic traits. We extracted phloem resistance values from the dataset compiled by

Liesche et al. (2017). For 22 woody angiosperm and gymnosperm species, we paired these data with the minimum mid-day leaf water potentials (Ψ_{\min}) and stem water potential thresholds for 50% declines in hydraulic conductance (stem Ψ_{50}) compiled by Choat et al. (2012) and Bartlett et al. (2016). The stem Ψ_{50} is a measure of xylem resilience to embolism (Tyree and Ewers 1991), with lower stem Ψ_{50} values supporting higher conductivity (ie, water supply) under drought. Thus, we predicted that this metric would correlate with phloem structural architecture as lower stem P50 should allow phloem architecture to support higher resistances as water is less limiting in these species. Since the data were not normal for phloem resistance, we used the spearman rank method to compute our test statistics. First, we tested the correlation between leaf Ψ_{\min} and phloem structural resistance to determine if phloem anatomy scales with the water stress of the plant. Next, we used a partial linear correlation to test if phloem resistance varies with stem Ψ_{50} while holding Ψ_{\min} constant. Statistical analysis was performed in R studio Version 1.2.5033 using the package ppcor.

Results

Phloem sap transport varies while sucrose export remains largely consistent under drought

Sugar export was constant for simulations with soil water potentials above the thresholds for runaway viscosity, and negligible for simulations below these thresholds (Fig. 2A). These thresholds depended strongly on the phloem hydraulic resistance, with the most resistant phloem ceasing to export sugar at the least negative water potentials (R4 and R5, Table 2).

Above these thresholds, sucrose export from the phloem was not impacted by soil water potential or phloem hydraulic resistance (Fig. 2A, R1 – R3). Over the course of these simulations, the sucrose concentration and turgor in the loading zone increased until phloem transport reached a steady-state equilibrium, where the mass of sucrose imported into the phloem equals the exported amount if $V_{\max U} \gg V_{\max L}$ (i.e., unloading is not constrained). Because loading rates are independent of water stress in apoplasmic-loading species, all simulations converged on the same equilibrium import and export rates, unless the model was unable to reach steady-state conditions. Simulations that reached steady state showed steadily increasing viscosity with declining soil water potentials (Fig. 2B). This caused a drop in phloem velocity by ~57% between the wettest and driest conditions for moderately resistant phloem (R3) (Fig. 2C), due to changes in the phloem resistance and source to sink pressure gradient. For the least resistant phloem (R1, circles; Fig. 2D), phloem pressure decreased with soil water potential, because the xylem water potential was decreasing at a greater rate than phloem osmotic potential (Fig. S1, green line). Pressures at the unloading area (base pressures) remained close to zero (all sugar was unloaded), signaling that unloading was not a limitation in these models. Increasing phloem resistance to moderate values (R2 – R3) increased the pressure required to overcome resistance to transport, producing higher phloem pressures overall and driving the trend of increasing pressures at lower soil water potentials.

The most resistant phloem (R4 and R5) was unable to achieve steady-state transport, which limited sucrose export at soil water potentials below -0.7 and -0.2 MPa, respectively. As Ψ_s became more negative, the concentration in the loading zone and, thus, the phloem viscosity and hydraulic resistance increased exponentially (Fig. 2B, asterisks). Overcoming greater resistance requires a higher loading zone turgor, which further increases the phloem concentration and, thus, the viscosity and hydraulic resistance. This can create a positive feedback loop where no loading zone concentration is able to overcome the resultant hydraulic resistance to transport sucrose to the unloading zone; as viscous resistance increases, so too does turgor pressure. In other words, declines in sucrose osmotic potentials outpaced declines in xylem water potential and caused phloem turgor to increase with water stress (Fig. S1, pink line). The most resistant phloem experienced this runaway viscosity effect even at relatively mildly negative soil water potentials (R4 and R5, Fig. 2B). Below these water potentials, sucrose import outpaced export and phloem viscosity, resistance, and turgor built until transport stopped entirely and the simulation was unable to return numerical results (indicated by asterisks in Figs. 2 & 3). Notably, the phloem transport variables always increased to intractable values immediately after reaching a loading zone pressure of 2.4 MPa (Fig. 2D). In these scenarios, sugar export from the phloem only proceeded for a small fraction of the 12-hour simulation. In sum, despite

phloem failure in the most resistant scenarios, sucrose export remained constant across a wide range of soil water potentials (i.e., 0 to -1 MPa) for moderate resistances.

Increasing the loading rate ($V_{\max L}$) by 40% increased the amount of sucrose exported by up to 40% in wet soil conditions when unloading was unlimited (i.e., all sugars reaching the unloading zone are immediately exported) (Fig. 3A). These scenarios appeared to show a trade-off in which higher transport rates are favored in wetter conditions, but in drier conditions, a lower rate is optimal to prevent runaway viscosity. Increasing the maximum loading rate increased the concentration and pressure to the 2.4 MPa threshold (Fig. S2, asterisks) for runaway viscosity and no steady state solutions were found under water-stressed conditions.

Faster loading produced runaway viscosity more easily in the scenarios where unloading was limited, with only the two lowest loading rates producing steady state solutions across a wide range of soil water potentials (Fig. 3B, black and gold lines). However, as $V_{\max U}$ approaches $V_{\max L}$ phloem export begins to lag what was being received in the loading area; we call this phenomenon an unloading limited scenario. In our unloading limited simulations, we set $V_{\max U}$ only 1% higher than $V_{\max L}$. Limiting unloading exacerbated runaway viscosity and reduced sucrose export for simulations with faster loading at even less negative soil water potentials (Fig. 3B).

Turgor regulation expands the operational range of phloem export under water stress

Halting sucrose loading at a turgor pressure of 2.4 MPa reduced the sensitivity of phloem transport to water stress and allowed sucrose transport to be maintained over a wider range of soil water potentials (Fig. 4). We selected 2.4 MPa as the loading threshold, since the model did not return steady state solutions at higher pressures (Fig. S2, asterisks). When the phloem was able to stop loading at this threshold, increasing the loading rate allowed sucrose export to be increased by at least 4% and up to 40% across all water potentials (Fig. 4A, red squares). Turgor regulation also eliminated the effects of the unloading limitation (Fig. 4B), with both unloading scenarios showing the same relationships between $V_{\max L}$ and sucrose export. Thus, a turgor-sensing loading mechanism (Fig. 4) allowed the phloem to operate effectively over a wider range of soil water potentials.

This feedback mechanism also allowed for a wider range of phloem resistance values. Under turgor pressure regulated loading, and at the highest $V_{\max L}$, we varied conduit resistance from 25% – 145% (R1 to R5; Fig. 5). The lowest resistance conduit maintained the highest sucrose unloaded across the entire range of soil water potentials (Fig. 5, circles). As the resistance of the conduit increased, the total amount of sucrose exported declined. At the maximum resistance (Fig. 5, inverse triangles), sucrose export was only 51% of the lowest resistance simulation, when run at the lowest soil water potential ($\Psi_s = -1$ MPa). Despite this reduction, the highest resistance phloem pathway was still able to export sugar while under turgor loading regulation (Fig. 5), while it would export none without this regulation (Fig. 2).

Evaluating the impact of stomatal control over sucrose export

Increasing stomatal conductance under water stress reduced sucrose export in some scenarios, but the impacts of the phloem loading kinetics and hydraulic resistance were considerably stronger (Fig. 6). First, we compared the effects of g_{\max} and $g_s \Psi_{50}$ on sugar export at the most extreme soil water potentials (i.e., -1 and -0.001 MPa) for different phloem hydraulic resistances (R1 & R4). A more negative $g_s \Psi_{50}$ (Fig. 6A) and higher g_{\max} (Fig. 6B) reduced sucrose export under water stress for the most resistant phloem (R4, diamonds). These stomatal traits are consistent with anisohydric plants, which keep stomata open despite drought conditions. However, the stomatal traits had no impact on export for less resistant phloem (R1, circles).

Overall, while increasing sucrose loading rate increased export by up to ~40%, changing stomatal behavior changed export by at most ~6%. However, the higher loading rate made the stomatal effects more important – i.e., export starts to decline with ‘riskier’ stomatal behavior. In addition, under well-watered conditions, neither phloem resistance nor stomatal behavior strongly affected sucrose export (Fig. 6C, D). Instead, increasing the loading rate had the greatest impact on export, and plants only suffered reduced export with

high phloem pathway resistances (red diamonds) while experiencing drought (Fig. 6 A, B).

Assessing the impact of phloem leakiness on phloem transport

Some sucrose may be leaked back out of the phloem membrane by diffusion, and we sought to determine whether this leakage is consequential for export efficiency. At modest soil water deficits (-0.5 MPa), the medium resistance scenario increased sucrose leakage by 80% compared to the lowest resistance (i.e., R1 vs. R3) (Fig. 7). However, this loss was negligible, as ~10 billion grams of sucrose would be exported before ~1 g leaked out of the loading phloem. Thus, the tendency of sucrose to leak out of the loading area by diffusion was determined to be of minimal consequence.

Meta-analysis of phloem architecture and plant hydraulic traits

We conducted a meta-analysis to evaluate relationships between phloem and hydraulic (xylem) architecture and the maximum water stress species typically experience in natural conditions. First, phloem resistance, calculated from sieve element anatomy, was not significantly correlated with maximum water stress, defined from the seasonal minimum mid-day leaf water potential (Ψ_{MD}) ($r_s = -0.241$, $N = 20$, $p [?] 0.05$). Further, phloem resistance was not significantly correlated with xylem embolism resistance (stem Ψ_{50}) when holding Ψ_{MD} constant, to account for the independent effects of environmental water stress on both variables ($r_s = -0.492$, $N = 20$, $p [?] 0.05$).

Discussion

Regulation of transporter kinetics were the main driver of maintaining sugar export in a variety of conditions, followed by changes in phloem pathway resistance, and finally changes in leaf stomatal traits. This importantly signals that stomatal control is decoupled from phloem transport in an apoplastic loader that regulates its turgor.

Sucrose export is maintained under drought when loading is stopped at high phloem pressures

Our model showed that setting a turgor threshold for loading prevented the buildup of sugars that causes runaway viscosity, reducing the impacts of water stress on sugar transport for highly resistant phloem. Turgor-regulated loading also allowed plants to benefit from faster loading in well-watered conditions without a trade-off on transport during drought. Consistent with these findings, previous work has shown that sucrose loading and unloading rates are sensitive to phloem turgor. Exposing the phloem to membrane-impermeable sugars (i.e., sorbitol or mannitol) reduces the apoplastic water potential outside the phloem and draws water from the phloem, lowering phloem turgor (Smith and Milburn 1980). These treatments upregulated sucrose loading in leaf discs from castor bean and unloading in seed coats for beans and root discs of beets (Smith and Milburn 1980, Patrick 1994, Bell and Leigh 1996).

Several mechanisms could allow for turgor-regulated transport by altering the abundance of sucrose transporters in the sieve tube plasma membranes. First, changes in phloem turgor could trigger a hormonal signaling cascade that alters transporter expression or post-translational modification (Patrick et al. 2001). Water stress impacts the transcript abundance of phloem loading sucrose transporters (Xu et al. 2018). Sucrose transporter activity is also highly dependent on post-translational modification. Sucrose loading proteins degrade from the plasma membrane in as little as four hours (Liesche et al. 2011) and loading activity can be altered by external conditions. For instance, ubiquitination of these proteins increases their degradation rate, while phosphorylation increases their affinity for sucrose in plants exposed to differing light (direction species dependent) (Xu et al. 2020). Second, the same mechanisms could regulate the proton motive force for sucrose transport (Patrick et al. 2001). In pea leaves, mannitol treatments reduced phloem pressure and the pH in the adjacent xylem, while increasing phloem sucrose uptake (Estruch 1989). This suggests that a lower turgor led the phloem to upregulate proton transporter activity to increase the proton motive force for sucrose loading. Finally, turgor could have direct biophysical impacts on loading, as increasing cell turgor compresses the plasma membrane, which could constrain the permeability of membrane-bound sucrose or proton transporters (Coster et al. 1977). We found that a phloem turgor sensing mechanism would

be essential for plants to maintain sugar transport in drought conditions, especially when their pathway resistance makes them more vulnerable to drought-induced failure. Thus, it will be critical for future studies to investigate the environmental catalysts behind the molecular regulation of sucrose transporters, and the consequences for phloem transport efficiency.

Recent modeling work also supports an important role for turgor-dependent membrane transport kinetics in phloem drought responses (Goeschel and Han 2020). This model assumes that the phloem downregulates unloading under water stress to prevent turgor from becoming negative in the unloading zone (Goeschel and Han 2020). However, this assumption was necessary for their simulations to capture observed responses of phloem concentration and velocity to water stress, which our model showed without turgor-dependent kinetics (Fig. 2). Without unloading regulation, the Goeschel model allows the unloading zone to reach negative turgor, so that a decline in plant water potential imposes the same decline in unloading zone turgor. This results in a maintenance of the source-to-sink pressure gradient and, thus, phloem concentration and velocity. Conversely, their equation to limit unloading maintained positive turgor at the unloading zone, causing the pressure gradient and consequently concentration and velocity to change with plant water potential. This change in velocity during drought is consistent with recent empirical measurements (Hesse et al. 2019, Dannoura et al. 2019). By contrast, our model prevents unloading zone turgor from falling below 0 MPa, since negative cellular turgor has never been recorded and has no clear biophysical meaning (Tyree 1976). If we remove this limitation, our results are similar to the simulations from Goeschel and Han (2020) without turgor-dependent unloading (data not shown). However, our model diverges from theirs by suggesting that turgor feedbacks on transport are important to prevent runaway viscosity under water stress, rather than a necessary condition to explain all phloem responses under normal conditions (Goeschel & Han 2020).

Phloem resistance is an important parameter for phloem failure, not export efficiency

Apart from the regulation of sucrose transporter kinetics, the next most influential trait in regulating translocation during drought is phloem pathway resistance. Using our higher phloem resistance values (R4–R5), we found that phloem could not operate under moderate drought stress of soil $\Psi = -1$ MPa without regulation of membrane transporters. Based on these findings, we hypothesize that phloem resistance within each organ of the plant has a functional window, past which phloem failure occurs. However, our meta-analysis shows that drought exposure and phloem resistance are not correlated across a range of woody species. Thus, although phloem architecture may scale within the source to sink phloem pathway of an individual plant, there is no evidence to support that water stress is a strong selector for phloem architecture (as stated by Liesche et al. 2017).

Modeled phloem resistance could increase by ~1 order of magnitude due to the occurrence of callose blockage at sieve plates in comparison to plates which are free of callose (Stanfield et al., 2019), or up to 20% due to p-protein agglomerations (Froelich et al. 2011). Insect attack (Hao et al. 2008) leads to callose blockages, and defoliation due to insects may reduce sieve tube diameters by up to 20% (Hillabrand et al. 2019) which would incur a large resistance to sap transport. The sieve tube has been hypothesized to compensate for these blockages by increasing its source-sink pressure differential (Knoblauch et al. 2014) or increasing phloem area and/or decreasing height by not growing as tall (Hölttä et al. 2006, 2009). Trees which cannot lower height instead increase phloem conducting area to lower resistance (Clerx et al. 2020) or change sieve plate allometry from source to sink (Savage et al. 2017). These structural remedies lower resistance and potentially alleviate the chances of phloem failure during drought as described in our model.

Previous models (including ours) largely assume that long distance pressure gradients drive Hagen-Poiseuille flow in the phloem, which may not be the case if sieve tubes are highly permeable to radial water flow (Sevanto 2014, Pfautsch et al. 2015). This distinction would change the mechanism for phloem failure under water stress, where phloem transport under extensive radial water exchange would fail from turgor loss and structural collapse in the sieve tubes instead of runaway viscosity (Sevanto 2014). In this scenario, a sieve tube with greater radial water exchange would exhibit greater water influx and faster sap flow under well-watered conditions, but greater water efflux and increased risk for turgor loss and cell collapse during drought. Sieve

tubes are lined with aquaporins along the source-sink pathway (Stanfield et al. 2017), which is consistent with this hypothesis, especially if aquaporins are unable to use gating mechanisms (e.g., Tornroth-Horsefield et al. 2006) to rapidly prevent phloem water efflux under drought. However, molecular regulation is key to prevent drought-induced phloem failure from either mechanism. Future experiments will need to distinguish these hypotheses through assimilate tracing during drought, followed by anatomical studies to determine if phloem cell collapse occurred.

Sucrose export is largely decoupled from stomatal behavior by molecular regulation of phloem loading

In contrast to previous work (Huang et al. 2018), our model showed that making stomatal behavior more conservative (isohydric) to limit declines in xylem water potential had little impact on sucrose export under drought in simulations with active molecular regulation of loading. The only time that an impact was found under drought conditions was when simulating a high phloem resistance; in these scenarios, changing from an extremely anisohydric strategy (e.g., $gs\Psi_{50} = -2.2$ MPa) to an extremely isohydric strategy (e.g., $gs\Psi_{50} = -0.2$ MPa) increased sucrose export by ~6%. However, this isohydric advantage was only possible through higher loading rates supported by pressure regulated sucrose transporters. If sucrose transporters could not be regulated in the phloem, then our model predicts stomata would have the impact of increasing the susceptibility to transport failure instead of adjusting the amount of sucrose exported. Huang et al. (2018) found that, for symplasmic loaders, maximizing the profit ratio between the sucrose exported by the phloem and produced by photosynthesis constrained gas exchange during drought. Isohydric simulations were more “cost effective” by closing the stomata to save water when the passively-loaded phloem was unable to generate the loading zone concentrations needed for export from the leaf. In simulations where loading was only proportional to carbon supply in the mesophyll, analogous to an apoplasmic loader, stomatal closure under drought was necessary to prevent runaway viscosity (Nikinmaa et al. 2013). Our findings demonstrate that downregulating sucrose loading is an effective alternative to stomatal closure, suggesting that phloem constraints on stomatal responses to drought could vary between symplasmic and apoplasmic loaders, or between apoplasmic loaders with different relationships between phloem pressure and transporter activity. Future work is needed to evaluate these hypotheses and the potential consequences for plant water and carbon fluxes under climate change.

Conclusions

Regulating phloem turgor via intermittent pausing of sucrose loading expanded the operational range of water potentials plants could experience and still maintain transport. This molecular regulation mechanism also largely decoupled phloem transport from stomatal behavior, except for extremely resistive phloem, by avoiding runaway viscosity and maintaining phloem function under drought. This mechanism also reduced the effects of a high phloem pathway resistance on transport, producing a large window of values with functionally identical sugar export rates. This finding leads us to hypothesize that ecosystem water stress has selected for resistance values within this window, to avoid excessive resistance and phloem failure. Future work will need to evaluate the runaway viscosity effect or turgor collapse hypotheses as the main driver of phloem failure and determine the degree of radial water permeability between the adjacent vascular tissues during drought.

Acknowledgements

RCS thanks the Rossi Postdoctoral and Katherine Esau Postdoctoral fellowships for funding.

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Table 1. Description of mathematical symbols used in the model.

Symbol	Definition	Value	Source
af	Apoplastic fraction inside the leaf	0.3	

Symbol	Definition	Value	Source
ags	Shape parameter relating $gs\Psi_{50}$ & Ψ_m	2.0	
A_{net}	Carbon assimilation through photosynthesis	-	
c_a	Atmospheric CO_2 concentration	400 Parts Per Million	
C_m	Concentration of sucrose in mesophyll	-	
$C_{pL,U}$	Concentration of sucrose in phloem loading (L) or unloading (U) area	-	
E_s	Rate of sucrose leaving phloem loading area	-	
f	Water flow through mesophyll (m) or xylem (x)	-	
Fp	Volumetric flow rate of phloem	-	
g_{max}	Maximum stomatal conductance	400 $mmol\ m^{-2}\ s^{-1}$ (default)	
g_s	Stomatal conductance	-	
$gs\Psi_{50}$	Water potential of mesophyll at 50% stomatal closure	-1.5 MPa (default)	
Km,x	Hydraulic conductance of mesophyll (m) or xylem (x)	-	
M_R	Michaelis-Menten constant for Rubisco	300 Parts Per Million	
K_{max}	Maximum hydraulic conductance of the leaf	20 $mmol\ m^{-2}\ s^{-1}\ MPa^{-1}$	
M_S	The affinity of the sucrose molecule to the SUT protein	3.3 mM	
$Leak$	The permeability rate of the sieve tube membrane to sucrose by diffusion	7.3e ⁻¹⁷ m/s (Default)	Edelman and Schoolar (1971)
LA	Leaf Area	47.4 cm^2	
$L_{sucL,U}$	Loading rate of sucrose per unit tissue area for the loading (L) or unloading (U) area	-	
mm	Molar mass of sucrose	342.3 $g\ mol^{-1}$	
F_p	Phloem flow rate	-	
$P_{L,U}$	Phloem pressure in loading (L) or unloading zone (U)	-	

Symbol	Definition	Value	Source
r	Radius of phloem conduit	1.8e^{-5} m	Esau 1962; Liesche et al. 2017
Rc	Gas constant	$8.3 \text{ m}^3 \text{ pa K}^{-1} \text{ mol}^{-1}$	
$R_{conduit}$	Resistance of the phloem conduit	-	
R_L	Leaf respiration rate	$0.25 \text{ } \mu\text{mol C m}^{-2} \text{ s}^{-1}$	
Rp	Structural resistance coefficient of the phloem	See Table 2	Borstlap and Schuurmans (2004)
RWC_m	Relative water content of the mesophyll	-	
Sl	Leakiness of sucrose in the phloem conduit	-	
S_m	Mass of sucrose in apoplastic space	-	
$S_{p,b}$	Mass of sucrose inside loading phloem (p) or unloading phloem (b)	-	
Sf	Mass fraction of sucrose in phloem sap	-	
t	Run time of model	12 hrs	
T	Temperature	293 K	
v	Viscosity of phloem conduit sap	-	
V_{cmax}	Maximum carboxylation rate	$40 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$	
V_{maxL}	Maximum rate of sucrose uptake (phloem loading)	$1.58\text{e}^{-7} \text{ mol m}^{-2} \text{ s}^{-1}$ (default)	
V_{maxU}	Maximum rate of sucrose export (phloem unloading)	$V_{maxL} * 3$ (no unloading limitation)	
VPD	Vapor Pressure Deficit of leaf	9.9e-3	
V_s	Velocity of sap in phloem conduit	-	
V_{sat}	Saturated water content of the leaf	33.3 mmol	
vw	Viscosity of pure water @ 200C	$1.0\text{e}^{-9} \text{ MPa s}$	
W	Water volume of the mesophyll (m), xylem (x), or leaf (l)	-	
W_p	Water volume inside phloem	$3.57\text{e}^{-11} \text{ m}^3$	
α	Shape parameter for determining mesophyll conductance	2	
Γ	Carbon Compensation Point	30 Parts Per Million	

Symbol	Definition	Value	Source
ϵ	Modulus of elasticity for mesophyll cell walls	15 MPa	
ρ	Density of phloem sap	-	
π_0	Osmotic potential of mesophyll at full hydration	-0.8 MPa	
π_μ	Osmotic potential of mesophyll	-	
$\pi_{\Lambda, \gamma}$	Osmotic potential of phloem @ loading (L) or unloading (U) zones	-	
$\pi_{\tau\lambda\pi}$	Turgor loss point	-	
$\Psi_{50,m}$	Mesophyll water potential at which 50% of the mesophyll conductance is lost.	-2.0 MPa	
Ψ_m	Water potential- mesophyll	-	
Ψ_s	Water potential- soil	See Table 2	
Ψ_x	Water potential - xylem	-	

Table 2. Parameters which varied during the simulations.

Symbol	Definition	Value
$Leak$	The permeability rate of the sieve tube membrane to sucrose by diffusion	$4.6e^{-17}$ m/s (L1, carrot callose) $2.92e^{-11}$ (L2, chloroplast membrane)
Rp	Structural resistance coefficient of the phloem	$1.58e^{20}$ (R1) $5.36e^{20}$ (R2) $6.30e^{20}$ (R3) $7.24e^{20}$ (R4) $9.13e^{20}$ (R5)
V_{maxL}	Maximum rate of sucrose uptake (phloem loading)	$1.58e^{-7}$ mol m ⁻² s ⁻¹ (100%) $1.74e^{-7}$ (110%) $1.90e^{-7}$ (120%) $2.06e^{-7}$ (130%) $2.22e^{-7}$ (140%)
V_{maxU}	Maximum rate of sucrose export (phloem unloading)	= V_{MaxL} *3 (no unloading limitation) = V_{maxL} *1.01 (unloading only 1% higher than loading)
Ψ_s	Water potential of soil	-0.001 - (-)1 MPa
g_{max}	Maximum stomatal conductance	20 – 400 mmol m ⁻² s ⁻¹
$gs\Psi_{50}$	Water potential of mesophyll at 50% stomatal closure	-0.2 – (-) 2.2 MPa

Figure Legends

Figure 1. Parameterization and conceptual schematic of the water and sugar transport model. The model inputs are shown in pink, while the outputs of the model are in red. The model first produces a xylem water potential based on the input soil water potential. This then generates a leaf mesophyll water potential that

then determines a stomatal conductance. The conductance of stomata (large green ovals) is modulated by stomatal properties such as G_{\max} or $G_s\Psi_{50}$, which then influences the amount of carbon that is assimilated through photosynthesis. The assimilated carbon then produces a sugar concentration in the mesophyll, which is then used for active phloem loading (symbolized by orange ovals). Phloem pressure in the loading zone was built by the osmotic potential overcoming the water potential of the xylem apoplast. Phloem outputs include sap velocity, viscosity, and pressure. Phloem characteristics which could be modified were structural resistance (R_{phloem}) or maximum sucrose import/export rate (V_{\max} load or V_{\max} unload). The ultimate result was the amount of sucrose that was exported into sink tissue.

Figure 2. Phloem output parameters modeled over varying soil water potentials and phloem resistance coefficients. (A) The amount of sucrose unloaded was consistent across soil water potentials, except under the two highest phloem structural resistances (R_4 & R_5). (B) The viscosity of the phloem sap increased as the soil water potential declined. At the highest pathway resistances (R_4 & R_5), the model no longer gave steady state solutions (asterisks) due to a run-away increase in viscosity. (C) The velocity of phloem transport declined with decreasing soil water potential, as well as increasing resistance. (D) Phloem pressures declined with decreasing soil water potential in the lowest resistance phloem, but began increasing with declining soil water at the higher resistances ($R_3 - R_5$). The phloem pressure in the unloading zone (base, X shapes) remained at near 0 MPa for the simulations tested which showed that there was not an unloading limitation. The maximum phloem pressure obtained that still researched a steady state was ~ 2.4 MPa (inverted triangles for R_5 resistance @ -0.3 MPa Ψ_s).

Figure 3. The impacts of the amount of sucrose unloaded to sink tissues over a soil dry-down while maximum loading rate (V_{\max}) was varied. (A) In an unloading unlimited scenario, whereby all sugar was removed from the unloading phloem area, increasing the loading rate had the consequence of increasing the sucrose output to the sinks under wetter soils (less negative soil water potentials). However, as the soil dried, the amount unloaded crashed to near zero as the run-away viscosity effect occurred. This was especially apparent in the higher loading rate scenarios, as phloem failure occurred in wetter soils. (B) The same simulation was run, except an unloading limitation was set such that sucrose started to accumulate in the unloading zone. In this scenario, only the slightly higher loading rate produced higher sucrose output over most soil water potentials, while the other loading rates exported significantly less. Overall, the effect of the unloading limitation was to make the phloem more susceptible to drought induced failure. In all simulations, the intermediate phloem resistance (R_3) was used.

Figure 4. Sucrose output over varying soil water conditions and sucrose export rate, with pressure sensitive loading active. In this simulation, loading is regulated and stops past a phloem pressure of 2.4 MPa and prevents the run-away viscosity event as evident in Figs. 2 & 3. (A) In an unloading unlimited scenario (see Fig. 3), sucrose output to sinks continues to increase with higher loading rates. Export amounts start to decline at lower soil water potentials, but never fall lower than the base loading rate export amount. (B) In an unloading limited scenario, similar results are shown to the unloading unlimited scenario. Overall, the effect of turgor regulated sucrose transporters were to prevent unloading limitation from becoming a bottleneck, and greatly lowered the impact of declining soil water potentials on the amount of sucrose exported from the phloem by preventing run-away viscosity. In all simulations, the intermediate phloem resistance (R_3) was used.

Figure 5. The amount of sucrose export over varying soil water potentials as a function of phloem pathway resistance, with turgor regulation and the highest V_{\max} (140%). Here turgor regulation allows phloem transport to operate over a wider array of soil water potentials and pathway resistances without resulting phloem transport failure due to the run-away viscosity effect. This simulation shows that a lower pathway resistance leads to the greatest amount of sucrose export, but that increased pathway resistance, while reducing export under drought, does not completely halt transport.

Figure 6. Sucrose output to sinks with varying stomatal properties ($G_s\Psi_{50}$; A, C) or (G_{\max} ; B, D), while varying loading rate (100% vs. 140%) or phloem pathway resistance (R_1 vs R_4). (A, B) In the two driest soil scenarios ($\Psi_s = -1$ MPa), stomatal properties did not alter the amount of sucrose exported under both

loading rates when phloem resistances were set to the lowest values (R1). However, once phloem resistance increased (R4), stomata that kept closed while dry (i.e., $G_s \Psi_{50} = -0.2$ or $G_{\max} = 40$) had a greater amount of sucrose exported than stomata that kept open (i.e., $G_s \Psi_{50} = -2.2$ or $G_{\max} = 400$). (C, D) When the soil was wetter, stomatal influences had much less of an influence than when the soil was dry, except for some slightly reduced export at the higher phloem resistance and loading rates (red triangles).

Figure 7. The fraction of sucrose leaked out of the loading area as a function of phloem pathway resistance. Although increased phloem pathway resistance did increase the amount of sucrose that leaked back out of the loading zone by diffusion, the overall fraction that did leak out in either scenario was miniscule.

Appendix A

The fluid viscosity of phloem sap was calculated using

$$v = v_w e^{(0.032Sf - (0.012Sf)^2 + (0.023Sf)^3)} \quad \text{Eqn 1A}$$

$$Sf = \frac{CpL^* \text{ mm}}{\rho} \quad \text{Eqn 2A}$$

$$\rho = \frac{CpL}{1000} 0.1256 + 1.0019 \quad \text{Eqn 3A}$$

where ρ and v are the density and viscosity of the phloem sap. These equations follow Jensen et al. (2013), where v is calculated from v_w , the viscosity of pure water at 200C, and Sf , the mass fraction of sucrose in the phloem (w/w), and ρ is calculated as a linear function of phloem sucrose concentration.











