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Optimal concentration for sugar transport in plants

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Vascular plants transport energy in the form of sugars from the leaves where they are produced to sites of active growth. The mass flow of sugars through the phloem vascular system is determined by the sap flow rate and the sugar concentration. If the concentration is low, little energy is transferred from source to sink. If it is too high, sap viscosity impedes flow. An interesting question is therefore at which concentration is the sugar flow optimal. Optimization of sugar flow and transport efficiency predicts optimal concentrations of 23.5 per cent (if the pressure differential driving the flow is independent of concentration) and 34.5 per cent (if the pressure is proportional to concentration). Data from more than 50 experiments (41 species) collected from the literature show an average concentration in the range from 18.2 per cent (all species) to 21.1 per cent (active loaders), suggesting that the phloem vasculature is optimized for efficient transport at constant pressure and that active phloem loading may have developed to increase transport efficiency.

1. Introduction

Flows of matter, energy and information are ubiquitous. Whether biological (vascular systems of plants and animals), geological (rivers, oceans and glaciers) or engineered (pipes, roads, electrical grids and the Internet), they serve the purpose of moving matter, energy or information from one place to another. Often, we find that such flows are constrained by a desire to either maximize the flow of material or minimize the energy dissipated by the flow. While a higher mass flow can be achieved by increasing the concentration, this happens at the expense of increased impedance which eventually causes the volume flow to decrease. At an intermediate optimum value of concentration the mass flow is therefore at a maximum, a phenomena well known in blood flow where the volume fraction of erythrocytes (haematocrit) that is optimal for transporting a maximum amount of oxygen is approximately 45% v/v [1] and in nectar feeding animals where the optimum concentration (approx. 30–50% wt/wt) depends on the drinking strategy employed [2]. Less is known, however, about the situation in plants where a concentrated solution of sugars dissolved in water transfer energy between distal parts of the plant.

Sugars produced by photosynthesis are transported in the phloem vascular system of plants. Transport is initiated in the leaves where sugars are either passively or actively loaded into phloem. In active loading species, the process is driven by membrane transporters and sugar polymerization and occurs against a sugar concentration gradient. However, in passive loading species, sugars move into the phloem without the use of metabolic energy by travelling down a concentration gradient from the mesophyll to phloem [3]. In the phloem, an aqueous solution of sugars, amino acids, proteins, ions and signalling molecules flow through a series of narrow elongated cylindrical cells, known as sieve tube elements, lying end-to-end forming a microfluidic network spanning the entire length of the plant. The solution moves with a flow speed $u \simeq 1 \text{ m h}^{-1}$ [4], and while the sieve tube radius a varies among species, it is often of the order $a \simeq 10 \text{ }\mu\text{m}$ [5]. Typically, the total solute concentration is approximately 20% wt/wt, and sugars, of which sucrose is the most abundant type, constitute 80–90% of this [6]. The flow in the phloem is driven by differences in hydrostatic pressure between source (leaves) and sink (e.g. roots or fruits) tissues believed to be generated by gradients in osmotic

potential between distal parts of the plant according to the Münch pressure flow hypothesis [7,8].

As in animals, it is likely to be physiologically favourable when a maximum amount of energy is transported through a given section of the plant vasculature. The existence of an optimum sugar concentration for this process was first proposed by Passioura [9] who argued that efficient transport of sugar requires concentrations in the range 14–35% based on increased viscous friction at high concentrations. Later, Lang [10] conducted a theoretical comparison of different sugars and sugar alcohols, and concluded that sucrose at $c \sim 25\%$ is the most advantageous substance to transport, since it is chemically stable, highly soluble and only generates a modest osmotic pressure when compared with other sugars, e.g. glucose and fructose. To our knowledge, however, no one has tested these predictions against measured values of phloem sap sugar concentrations. Also, it is not clear how the relation between driving pressure and sugar concentration influence the predictions given above, or how phloem loading mechanisms may be related to transport efficiency.

In this paper, we derive general criteria for determining the concentrations that maximize the mass flow and efficiency of sugar transport in plants. Our analysis thus extends the work of Passioura [9] and Lang [10] by providing a general framework for evaluating the efficiency of sugar transport in plants. We compare the predictions of the model with measured sugar concentrations from more than 50 experiments (41 species) collected from the literature, and consider the effects of sugar loading mode.

2. Mathematical model

Sugar is produced in the leaves during photosynthesis and is then transferred to the phloem vascular system by an either active or passive loading mechanism as discussed in §1. Once loaded, the mass flow of sugar J through a given section of phloem tissue can be expressed in terms of the volumetric flow rate Q of phloem sap, the sugar mass fraction (concentration) c and the density ρ of the solution

$$J = Q\rho c. \quad (2.1)$$

The magnitude of the Reynolds number $Re = \rho ua/\eta \approx 10^{-3}$ allows us to assume a laminar Poiseuille pipe flow where the volume flow rate Q is determined by the geometry of the phloem channel, the pressure differential driving the flow Δp and the viscosity of the sugar solution η

$$Q = X \frac{\Delta p}{\eta}. \quad (2.2)$$

Here, X is a geometric parameter which for cylindrical phloem cells of radius a and length L is given by $X = \pi a^4/(8L)$. In a plant of length $L = 10$ m and sieve tube radius $a = 10$ μm , the geometric factor $X \approx 3.9 \times 10^{-22}$ m^3 .

In general, both the pressure differential Δp , density ρ and viscosity η in equations (2.1) and (2.2) depend on the sugar concentration c . The viscosity and density are known functions of concentration, plotted in figure 1*a,b*. The pressure differential Δp required to drive the phloem flow is of the order of 0.1–1 MPa and is believed to be generated by osmotic pressure gradients [7,8]. Other mechanisms have been proposed, e.g. electroosmotic or protoplasmic streaming, but these have been less successful in explaining experimental data [12]. Notwithstanding the mechanism driving the flow,

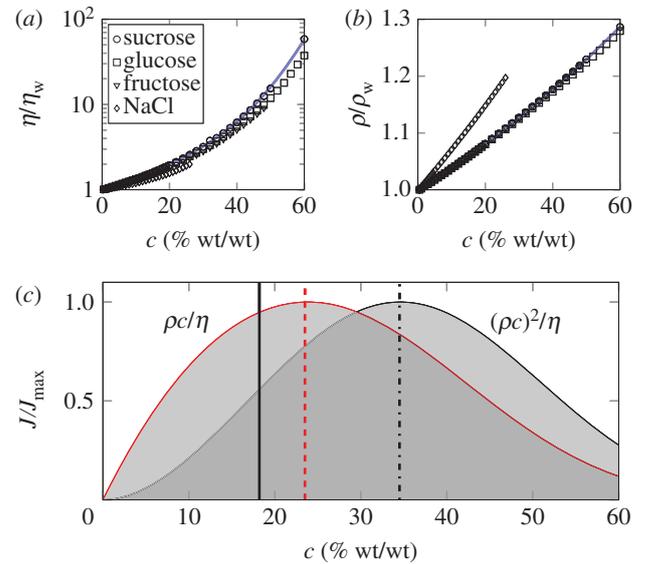


Figure 1. Optimal concentration for sugar transport in plants. (a) Viscosity η in units of the viscosity of pure water η_w plotted as a function of mass fraction c for sucrose (circles) and other solutes as indicated in the legend. Solid line is fit to sucrose data. (b) Density ρ in units of the density of pure water ρ_w plotted as a function of mass fraction c for sucrose (circles) and other solutes as indicated in the legend of (a). Solid line is fit to sucrose data. Data in (a) and (b) are from [11], see appendix A. (c) Normalized mass flow curves $J \propto \rho c/\eta$ (equation (2.3)) and $J \propto (\rho c)^2/\eta$ (equation (2.4)) plotted as a function of sucrose concentration c with η and ρ taken from data fits in (a) and (b). The maximum rates of sugar transport are found at 23.5% for constant pressure $\Delta p = \Delta p_0$ (dashed vertical line) and at 34.5% for variable pressure $\Delta p = RT\rho c/M$ (dash-dotted vertical line). The average value of the sugar concentrations reported in table 2 (18.2%) is indicated by the solid vertical line. (Online version in colour.)

we divide the problem into two categories: those in which the pressure $\Delta p = \Delta p_0$ does not depend on sugar concentration, and those in which pressure does depend on sugar concentration, $\Delta p = \Delta p(c)$. An example of a concentration-dependent pressure is found in the classical interpretation of the Münch hypothesis [13–19], where the pressure differential Δp is the osmotic pressure difference associated with the molar sugar concentration gradient $\Delta(\rho c)/M$ between source and sink, i.e. $\Delta p(c) = RT\Delta(\rho c)/M$, where M is the molar mass of the solute, R the gas constant and T the absolute temperature. Here, we use the van't Hoff value $RT\Delta(\rho c)/M$ for the osmotic pressure, which is valid only for dilute (ideal) solutions. If sugars are unloaded effectively at the sink region, it is reasonable to take the sugar concentration difference to be approximately given by the characteristic concentration itself $\Delta(\rho c) \approx \rho c$ and so the pressure is proportional to concentration $\Delta p(c) = RT\rho c/M$. For simplicity, we do not consider variations in viscosity along the pathway associated with this estimate of the osmotic driving pressure.

From equations (2.1) and (2.2), we can finally write the sugar flow rate J as

$$J = X\Delta p_0 \frac{\rho c}{\eta}, \quad (2.3)$$

if the pressure $\Delta p = \Delta p_0$ is constant, and as

$$J = X \frac{RT(\rho c)^2}{M\eta}, \quad (2.4)$$

if the pressure $\Delta p(c) = RT\rho c/M$ is proportional to concentration.

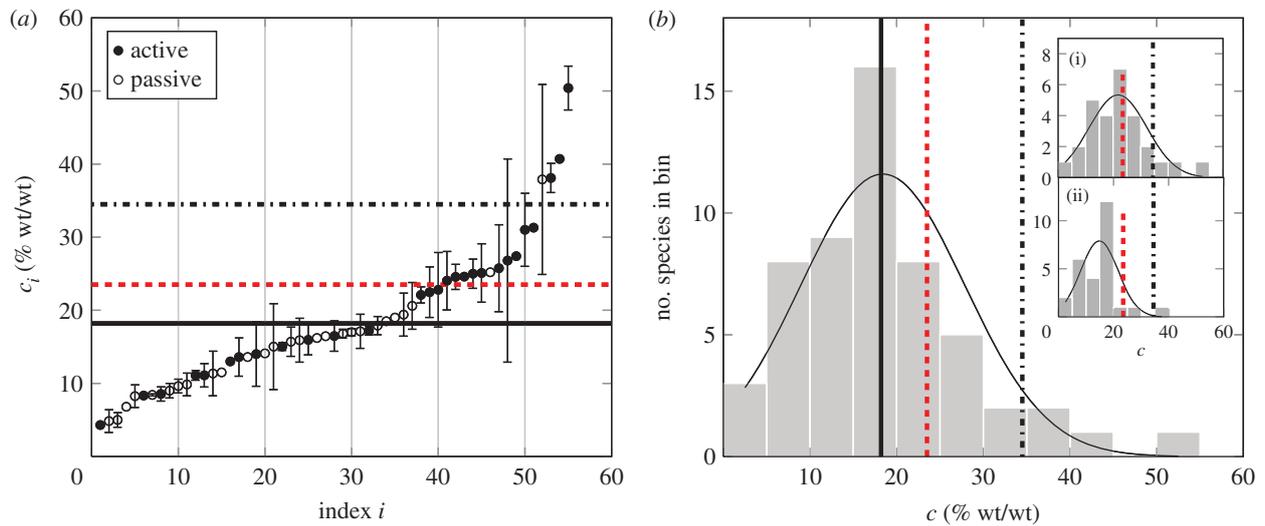


Figure 2. Sugar concentration in phloem sap. (a) Sugar concentration c_i for species number i as listed in table 2. Legend indicates phloem loading type. Error bars show standard deviation or range of concentrations reported. (b) Histogram showing distribution of sugar concentrations from (a). Inset shows histogram (i) for active and (ii) passive loaders. Thin solid lines are normal distributions fitted to the histograms as a guide to the eye. In (a,b), the thick solid line indicates the mean value 18.2% of phloem sap concentrations given in (a) (table 1). Dashed line indicates the optimum concentration 23.5% for flows driven by constant pressure while the dash-dotted lined indicates the optimum concentration 34.5% for flows driven by concentration-dependent pressure. (Online version in colour.)

2.1. Maximizing flux

To optimize sugar mass flow, we must maximize the expressions in equations (2.3) and (2.4), i.e. find solutions of $\partial J/\partial c = 0$ and $\partial^2 J/\partial c^2 < 0$. As shown in figure 1c, the optima for these two cases occur at 23.5 per cent and 34.5 per cent, respectively. Optimal concentrations for the case where the pressure Δp is linear combination of the forms given above (i.e. $\Delta p = \Delta p_0 + RT\rho c/M$) must necessarily lie between these two values.

2.2. Maximizing efficiency

Having established the concentration that maximizes sugar mass flow, we will now consider the efficiency of the transport process. The energy E transferred per unit time by the movement of sugars between distal parts of the plant depends on the sugar flow J and energy content k per unit mass of solute $E = kJ = kX\Delta p\rho c/\eta$. Several factors contribute to the energetic cost of transporting sugar: maintaining the pressure gradient, building and preserving the vasculature, loading and unloading of solutes, and power dissipated by the viscous flow. While not all these are easily quantifiable, the viscous power $p = X\Delta p^2/\eta$ dissipated by the flow is known to strongly influence transport in other biological systems [20–22]. Assuming this will be the dominant source of loss, the net energy \bar{E} available at the sugar sink is therefore

$$\bar{E} = E - P = E \left(1 - \frac{\Delta p}{k\rho c} \right). \quad (2.5)$$

The pressure difference Δp required to drive the observed flow speeds ($u \simeq 1 \text{ m h}^{-1}$, [4]) is of the order of $\Delta p = 0.1 - 1 \text{ MPa}$. With $c = 0.2$ and $k = 10^4 \text{ J g}^{-1}$ [23], we find $\Delta p/(k\rho c) \simeq 10^{-3}$ so the energy lost to viscous friction is almost negligible (i.e. \bar{E}), and optimizing the net energy transfer \bar{E} in equation (2.5) is equivalent to maximizing J in equations (2.3) and (2.4).

Similarly, we can define the efficiency $\phi = \bar{E}/P$ as the amount of useful energy the plant can extract from the flow

Table 1. Average phloem sugar concentration (in %wt/wt) determined from the data in table 2 for all species, active loaders and passive loaders. In the species averaged column, the reported concentrations for each species were averaged across studies before taking the total average. In the including repeated species column, the reported concentrations for each species were not averaged before taking the total average.

	species averaged	including repeated species
all species	18.2	18.4
active loaders	21.1	21.8
passive loaders	15.4	14.9

per unit energy spent on the transport process. Using the estimates given above, we find $\phi/P \simeq 10^3 \gg 1$ and conclude that optimizing the efficiency ϕ is equivalent to optimizing the flow J in equations (2.3) and (2.4).

3. Material and methods

3.1. Phloem sap sugar concentration data

Phloem sap sugar concentration from 41 species (55 experiments) was collected from the literature. The data are listed in table 2 and plotted in figure 2. Phloem sap is challenging to collect because disruption of the cell membrane releases the hydrostatic pressure inside a sieve tube, which can potentially damage intracellular structures [24], block phloem transport [25] and contaminate fluid samples with apoplastic material. Currently, there are two commonly used methods for collecting phloem sap: bleeding and stylectomy. Bleeding is achieved by making diagonal incisions into the bark of woody species [26] or by transversely cutting through entire organs, such as petioles, pedicels or branches [27]. This technique is primarily used on trees and a handful of herbs that readily bleed from incisions (e.g. *Ricinus communis* [28], cucurbits [29] and some legumes [30]).

Table 2. Phloem sap sugar concentration data. Sugar mass fraction c_i and standard error/range δc_i obtained from reference Ref I using either bleeding (b) or stylectomy (s) protocols, as indicated in column PC. The protocol for determining the loading mechanism (PLM) was based on studies examining plasmodesmata (1), autoradiographs (2), leaf sugar concentration (3), osmolality of leaf sap (4), incipient plasmolysis (5) and sugar transporters (6) from reference Ref II. Whenever possible, species (S)- and genus (G)-level studies were used to infer loading type. For several species loading type was determined based on plant family (F). Index i refers to the position of the species along the coordinate axis in figure 2a.

i	species	common name	c_i	(δc_i)	PC	ref I	loading mechanism	PLM	ref II
1	<i>Brassica oleracea</i> L.	cabbage	4.3		(b)	[53]	active	G(1)	[54]
2	<i>Salix</i> L. sp.	willow	4.8	(1.6)	(s)	[55]	passive	G(1,2,3,5)	[56]
3	<i>Salix viminalis</i> L.	basket willow	5.0	(1.0)	(s)	[37]	passive	G(1,2,3,5)	[56]
4	<i>Humulus lupulus</i> L.	common hops	6.8		(b)	[7]	passive	F(1)	[54]
5	<i>Ricinus communis</i> L.	castor bean	8.3	(1.6)	(b)	[57]	passive	S(1)	[54]
6	<i>Triticum aestivum</i> L.	wheat	8.3		(s)	[39]	active	S(6)	[58]
7	<i>Salix acutifolia</i> Willd	littletree willow	8.4	(0.1)	(s)	[36]	passive	G(1,2,3,5)	[56]
8	<i>Fraxinus americana</i> L.	white ash	8.5	(1.0)	(b)	[29]	active	S(1,2,3)	[59]
9	<i>Ricinus communis</i> L.	castor bean	9.0	(1.0)	(b)	[28]	passive	S(1)	[54]
10	<i>Platanus occidentalis</i> L.	American sycamore	9.6	(0.9)	(b)	[29]	passive	G(1,2,3)	[59]
11	<i>Ricinus communis</i> L.	castor bean	9.9	(1.6)	(b)	[60]	passive	S(1)	[54]
12	<i>Fraxinus pennsylvanica</i> Marshall	green ash	11.1	(0.7)	(b)	[29]	active	G(1,2,3)	[59]
13	<i>Arabidopsis thaliana</i> (L.) Heynh.	mouse-ear cress	11.1	(1.6)	(s)	[61]	active	S(6)	[62]
14	<i>Ricinus communis</i> L.	castor bean	11.4	(3.1)	(b)	[45]	passive	S(1)	[54]
15	<i>Pinus sylvestris</i> L.	scots pine	11.5		(b)	[7]	passive	G(1)	[54]
16	<i>Medicago sativa</i> L.	clover	13.0		(s)	[63]	active	G(1)	[54]
17	<i>Robinia pseudoacacia</i> L.	black locust	13.6	(2.6)	(b)	[64]	active	G(1)	[54]
18	<i>Quercus pedunculata</i> Willd.	pedunculate oak	13.6		(b)	[7]	passive	G(1,2,3,4)	[3]
19	<i>Lupinus albus</i> L.	white lupine	14.0	(4.4)	(b)	[65]	active	G(1)	[54]
20	<i>Arenga pinnata</i> (Wurmb) Merr.	sugar palm	14.1		(b)	[66]	passive	F(1)	[54]
21	<i>Salix viminalis</i> L.	basket willow	15.0	(5.9)	(s)	[67]	passive	G(1,2,3,5)	[56]
22	<i>Nicotiana glauca</i> Graham	tree tobacco	15.1	(0.6)	(b)	[68]	active	G(1,2,3,4)	[3]
23	<i>Carpinus betulus</i> L.	European hornbeam	15.7	(2.0)	(b)	[7]	passive	S(1)	[54]
24	<i>Salix exigua</i> Nutt.	narrowleaf willow	15.9	(3.0)	(s)	[40]	passive	G(1,2,3,5)	[56]
25	<i>Lactuca sativa</i> L.	lettuce	16.0	(2.1)	(s)	[32]	active	F(1,2,3,4)	[3]
26	<i>Castanea sativa</i> Mill.	European chestnut	16.2		(b)	[7]	passive	G(1)	[54]
27	<i>Oryza sativa</i> L. var. Towada	rice	16.5	(0.1)	(s)	[69]	passive	S(1,3,6)	[70]

(Continued.)

Table 2. (Continued.)

<i>i</i>	species	common name	c_i	(δc_i)	PC	ref I	loading mechanism	PLM	ref II
28	<i>Robinia pseudoacacia</i> L.	black locust	16.5	(2.1)	(b)	[29]	active	G(1)	[54]
29	<i>Tilia platyphyllos</i> Scop.	large-leaved lime	16.8	(0.6)	(b)	[64]	passive	G(1,2,3,4)	[3]
30	<i>Quercus borealis</i> Michx. f.	northern red oak	17.0	(0.5)	(b)	[64]	passive	G(1,2,3,4)	[3]
31	<i>Quercus rubra</i> L.	northern red oak	17.1	(2.3)	(b)	[7]	passive	S(1,2,3,4)	[3]
32	<i>Triticum aestivum</i> L.	wheat	17.2	(0.6)	(s)	[71]	active	S(6)	[58]
33	<i>Acer platanoides</i> L.	Norway maple	17.9	(1.2)	(b)	[64]	passive	G(1,2,3,4)	[3]
34	<i>Tilia</i> L. sp.	basswood	18.5		(b)	[7]	passive	G(1,2,3,4)	[3]
35	<i>Tilia parvifolia</i> Ehrh.	small-leaved lime	19.0		(b)	[7]	passive	G(1,2,3,4)	[3]
36	<i>Oryza sativa</i> L. var. Towada	rice	19.4	(2.9)	(s)	[38]	passive	S(1,3,6)	[70]
37	<i>Acer pseudoplatanus</i> L.	sycamore maple	20.6	(3.2)	(b)	[7]	passive	G(1,2,3,4)	[3]
38	<i>Fraxinus americana</i> L.	white ash	22.1	(1.1)	(b)	[72]	active	S(1,2,3)	[59]
39	<i>Brassica nigra</i> (L.) W. D. J. Koch	black mustard	22.5	(3.5)	(s)	[73]	active	G(1)	[54]
40	<i>Robinia pseudoacacia</i> L.	black locust	22.8	(5.1)	(b)	[7]	active	G(1)	[54]
41	<i>Lupinus mutabilis</i> Sweet	Tarwi	24.0	(4.0)	(b)	[30]	active	G(1)	[54]
42	<i>Avena sativa</i> L.	common oat	24.6	(1.7)	(s)	[74]	active	F(1)	[54]
43	<i>Spinacia oleracea</i> L.	spinach	24.6		(s)	[75]	active	S(3)	[76]
44	<i>Fraxinus americana</i> L.	white ash	25.0	(2.0)	(b)	[64]	active	S(1,2,3)	[59]
45	<i>Eucalyptus globulus</i> Labill.	Tasmanian bluegum	25.1	(4.0)	(b)	[77]	active	G(1,3)	[54,78]
46	<i>Acer platanoides</i> L.	Norway maple	25.2		(b)	[7]	passive	G(1,2,3,4)	[3]
47	<i>Zea mays</i> L.	maize	25.8	(6.0)	(s)	[79]	active	S(1,3,5)	[80,81]
48	<i>Tanacetum vulgare</i> L.	tansy	26.8	(13.9)	(s)	[45]	active	F(1,2,3,4)	[3]
49	<i>Zea mays</i> L.	maize	27.4		(s)	[82]	active	S(1,3,5)	[80,81]
50	<i>Hordeum vulgare</i> L.	barley	31.0	(5.0)	(s)	[83]	active	S(1,3)	[76,84]
51	<i>Lolium perenne</i> L.	ryegrass	31.3		(s)	[31]	active	S(1,3,5,6)	[31,85]
52	<i>Prunus persica</i> L.	peach	37.9	(13)	(s)	[86]	passive	S(1,2,3)	[59]
53	<i>Solanum tuberosum</i> L. cv. Desiree	potato	38.1	(2.0)	(s)	[87]	active	S(1,2,3)	[59]
54	<i>Zea mays</i> L.	maize	40.7		(s)	[81]	active	S(1,3,5)	[80,81]
55	<i>Solanum tuberosum</i> L. cv. Desiree	potato	50.4	(3.0)	(s)	[46]	active	S(1,2,3)	[59]

Bleeding techniques that rely on EDTA and measurements on cucurbits were not included in this meta-analysis because of debate over the purity of their exudates [31–34]. Stylectomy [35] uses the severed stylets (mouthparts) of phloem-feeding insects (e.g. aphids [36,37], planthoppers [38,39] and scale insects [40]). These insects feed on individual sieve elements without disrupting phloem transport, thereby creating a direct tap into functional sieve tubes with their stylets [41]. Once the stylets are cut, droplets of relatively pure phloem sap can be collected with capillary tubes.

Both bleeding and stylectomy have advantages and disadvantages as techniques for sampling phloem sap. Bleeding techniques are relatively simple to use but they are destructive and susceptible to contamination by apoplastic material and intracellular contents of neighbouring cells [31,42]. Samples produced by bleeding are also subject to dilution over time by water that is osmotically attracted to the incision [43], and may thus only provide a lower limit on the actual phloem concentration. Conversely, stylectomy yields more pure phloem sap because the sampling is less invasive, and although aphids can alter the amino acid composition of phloem exudates [44] there is no evidence that these changes directly affect sugar concentration. The disadvantage of this technique is that it is technically challenging and is susceptible to the effects of evaporation because of the small size of the exudate droplets [45]. Furthermore, it can only be used in plant species associated with sap-feeding insects [35], which could lead to biases in sample collection if these insects prefer certain types of plants. For example, research on transgenic potatoes [46] and in artificial systems [47] suggests that aphids may selectively feed on sieve tubes based on their high sugar concentration relative to neighbouring cells. If this is a common mechanism employed by sap-feeding insects, they may have a stronger affinity towards active loading plant species.

A least-squares regression model was used to examine whether there were systematic differences in the phloem sap sugar concentrations in samples collected by stylectomy and bleeding, and between passive and active loading species. The model considered the effect of each bivariate factor individually and their interaction, and multiple comparisons of all combinations of these variables were conducted using Tukey's HSD ($\alpha = 0.05$). To avoid oversampling of common species, phloem sugar concentrations for each species were averaged across studies. For averages of the different groups, see table 1.

4. Results and discussion

We have derived a general framework for determining sugar concentrations that optimizes phloem transport under different conditions, and a relatively complete physical picture of the process has emerged. First and foremost, the optimal sugar concentration for a given plant depends only on how the pressure differential Δp is maintained. At constant pressure, we find that the mass flow of sugar is optimal when $c = 23.5\%$, in agreement with the predictions by Passioura [9] and Lang [10]. We have further shown that optimizing transport efficiency in this case leads to a similar prediction. On the other hand, if the pressure is proportional to the concentration, we have shown that the transport efficiency and mass flow of sugar is optimal when $c = 34.5\%$.

From figure 1*a,b*, we observe that the mass fraction c is the major determinant of both viscosity and density of aqueous sugar solutions. It follows that all sugars which are dissolvable in water to a concentration of at least 23.5 per cent yield approximately the same mass flow at constant pressure, because equation (2.3) does not depend on the molar mass M . When the flow is driven primarily by the osmotic pressure

of the sugar itself (equation (2.4)), however, light sugars such as fructose or glucose should have a distinct advantage over sucrose, since the sugar flow scales $J \propto M^{-1}$. To argue why sucrose is nevertheless the most abundant compound, one must therefore consider other effects, such as the chemical properties of sugars and sugar alcohols discussed by Lang [10], see §1.

The optimal concentrations discussed above provide a rationale for the observation that the mean sugar concentration in the phloem sap of the plants considered in this study is 18.2 per cent, which is closer to the predicted optimum concentration for sugar transport under constant pressure (figure 2 and table 1). When considering active sugar loaders separately the trend is even more clear: average concentration 21.1 per cent (figure 2*b(i)*). Interestingly, the fact that the observed values cluster around the optimum value for constant pressure suggests that the pressure difference Δp driving the flow may be constant, and consequently does not scale with the sugar concentration c . Under a scenario where translocation is driven by osmotic pressure generated by both sugars and ions, this implies that changes in the sugar concentration c are balanced by additional terms in the pressure, i.e. $\Delta p = f(c) + RT\rho c/M = \text{const.}$, such that the total pressure Δp remains constant. This can be achieved by active loading and unloading of potassium ions [48–51] and may explain why pressure does not scale with plant size [52].

Together, loading type and sampling method appear to impact the sugar concentrations measured in the phloem (ANOVA, $F = 3.37$, $p = 0.028$). This effect is due to the significant difference (Tukey HSD, $t = 2.94$, $p = 0.027$) between active loaders measured with stylectomy (24.3 ± 2.3) and passive loaders sampled by bleeding (15.2 ± 2.1). Although there is not a direct interaction between loading type and sampling method (ANOVA, $F = 1.95$, $p = 0.17$), these factors are potentially confounding because of bias in the sampling method used for each loading type (table 2). As a result, differences in the sampling methods (described in §3*a*) may partially explain the higher sugar concentrations observed in the active loaders compared with the passive loaders (figure 2*b(i),(ii)*). However, if the higher sugar concentrations measured in the active loaders are not an artefact of differences in these methods, the data would suggest that active loaders achieve more optimal concentrations for transport than passive loading species, thus providing a new perspective on recent discussions regarding the potential advantages of using an active loading method [52,88]. If this loading method allows for more efficient transport, it may facilitate a faster growth habit and explain why active loaders tend to be faster growing herbaceous species and passive loaders tend to be slower growing trees [89]. Plants with the highest reported sugar concentration are broadly characterized by rapid growth, and several of those presented here are crop plants (e.g. potato ($c = 50.4\%$) and maize ($c = 40.7\%$)). These extreme concentrations may thus be an artefact of selective breeding.

Several caveats are in order, however. The optimal concentration predicted here might differ from that observed in nature due to the limited availability of light, water and nutrients. Although some plant species maintain fairly constant sugar concentrations in their phloem (e.g. *Ricinus*), other species appear to exhibit diurnal and seasonal changes in sap chemistry. In the autumn, growth cessation leads to reduced sink activity, which coincides with a 1.5–5-fold increase in the sugar content of the phloem exudates of some deciduous [40,67,90] and evergreen species [91]. In these species, the phloem may serve as a

reservoir of carbon that plants can access without the use of temperature-sensitive enzymes [92]. Higher sugar concentrations may also prevent desiccation during extracellular freezing and facilitate supercooling during the winter [40]. On a smaller temporal scale, sugar concentrations in the phloem can increase by 25–160% between the night and the day [45,71,87] and can change in response to disturbance such as defoliation [31]. Therefore, some of the variation observed in phloem sugar concentration could be the result of differences in growth and sampling conditions used in each study. Optimal concentrations suggested by models thus still need to be carefully scrutinized in attempts to understand the evolutionary implications of sugar loading strategies.

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