Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Sieve-element differentiation and phloem sap contamination

Michael Knoblauch[1], Winfried S Peters[1], Karen Bell[2], Timothy J Ross-Elliott[1,3] and Karl J Oparka[2]

Sieve elements (SEs) degrade selected organelles and cytoplasmic structures when they differentiate. According to classical investigations, only smooth ER, mitochondria, sieve element plastids, and, in most cases, P-proteins remain in mature SEs. More recent proteomics and immunohistochemical studies, however, suggested that additional components including a protein-synthesizing machinery and a fully developed actin cytoskeleton operate in mature SEs. These interpretations are at odds with conventional imaging studies. Here we discuss potential causes for these discrepancies, concluding that differentiating SEs may play a role by 'contaminating' phloem exudates.

Addresses
[1] School of Biological Sciences, Washington State University, Pullman, WA 99163, USA
[2] Institute of Molecular Plant Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, UK
[3] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Corresponding author: Knoblauch, Michael (knoblauch@wsu.edu)

Current Opinion in Plant Biology 2018, **43**:43–49

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Noel Michele Holbrook** and **Michael Knoblauch**

https://doi.org/10.1016/j.pbi.2017.12.008

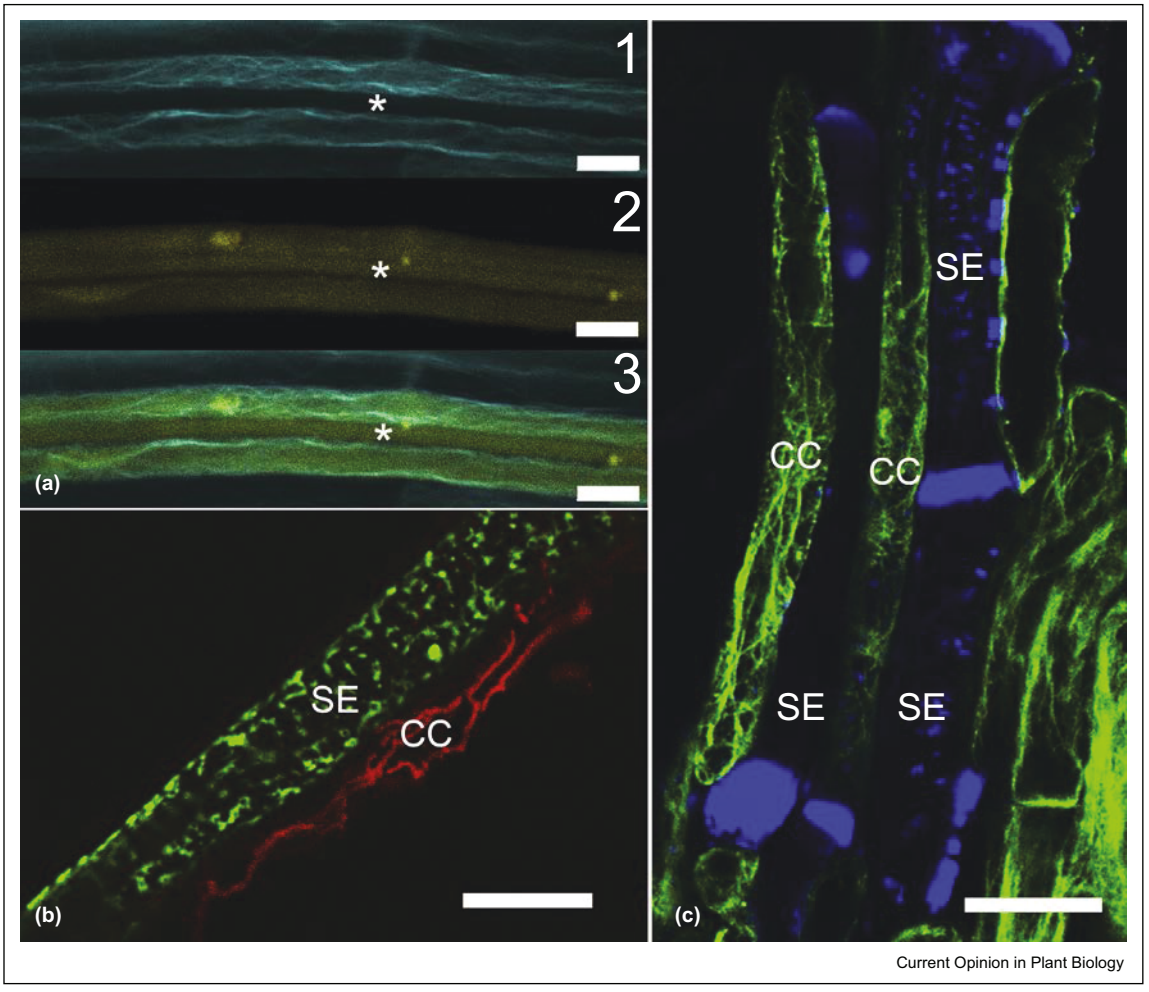
Introduction

In agreement with the function of sieve elements (SEs) in long-distance transport which favors an unobstructed tube with minimal flow resistance, differentiating SEs undergo partial autolysis [1]. It may take only minutes for a young SE to lose its nucleus, vacuole, and Golgi apparatus, as it connects to the existing sieve-tube system [1,2•]. Historically, extensive electron microscopy studies of mature SEs detected mitochondria, smooth ER, SE-plastids, and phloem-specific proteins (P-proteins), but no cytoskeleton. Except for forisomes, P-protein bodies involved in reversible sieve tube occlusion in legumes [3], the functions of P-proteins [4] and SE plastids are unknown. Application of correlative microscopy and super-resolution imaging [5–7] showed that, unlike P-proteins, membrane-bound organelles are restricted to a thin parietal layer in functional SEs. Gentle preparation and flash-freezing methods enabled the identification of protein clamps linking some of the SE organelles to each other and to the cell membrane [8,9]. Despite the wealth of evidence speaking against the presence of ribosomes and a cytoskeleton in sieve tubes (reviewed in [10]), a functional actin filament network [11,12] and protein synthesis machinery [13] were postulated to exist in mature SEs. Such uncertainties concerning SE structure are partially due to methodological challenges that derive from, first, the high turgor pressure in sieve tubes usually in the range of 1–2.5 MPa, second, the embedding of SEs in parenchyma and companion cells (CCs), hindering isolation of 'pure' sieve tubes, and third, the systemic nature of the sieve tube system in which local artefacts may be transmitted over long distances [7]. Here we discuss the contamination of sieve tubes with material from differentiating SEs as an additional factor potentially responsible for the contradictory findings.

Do mature SEs have an actin cytoskeleton?

Monomeric actin and various actin-binding proteins are found routinely in phloem exudates [13–17]. The debate about their significance in SEs was stimulated greatly by Hafke *et al.* [11], who were cited as having 'unequivocably shown that SEs contain a fully developed actin network' [18]. Unsurprisingly, this fostered new hypotheses on the actin cytoskeleton in relation to phloem function and plant-pathogen interactions [12,19,20]. Hafke *et al.* [11] provided two lines of evidence for an actin network in mature SEs. First, microinjection of fluorescent phalloidin into sieve tubes resulted in labeling of an extensive meshwork in the periphery of the SEs. Unfortunately, tagged phalloidin fluoresces independently of any association with actin and may bind to unrelated targets including forisomes, as noticed in [11]. Thus, phalloidin labeling lacks actin-specificity in sieve tubes. Moreover, the structure of the putative, phalloidin-labeled actin cytoskeleton looked remarkably similar to the sieve-tube ER and P-protein meshworks described by other authors (compare Figure 1 in [11] with our Figure 1b, Figure 2 in [9], or Figure 2 in [21]). Second, immunocytochemical TEM images showed labeling in the periphery of SEs (Figure 2 in [11]). However, the putative actin filaments lack the constant diameters and distinct fibrillar structure that

Figure 1



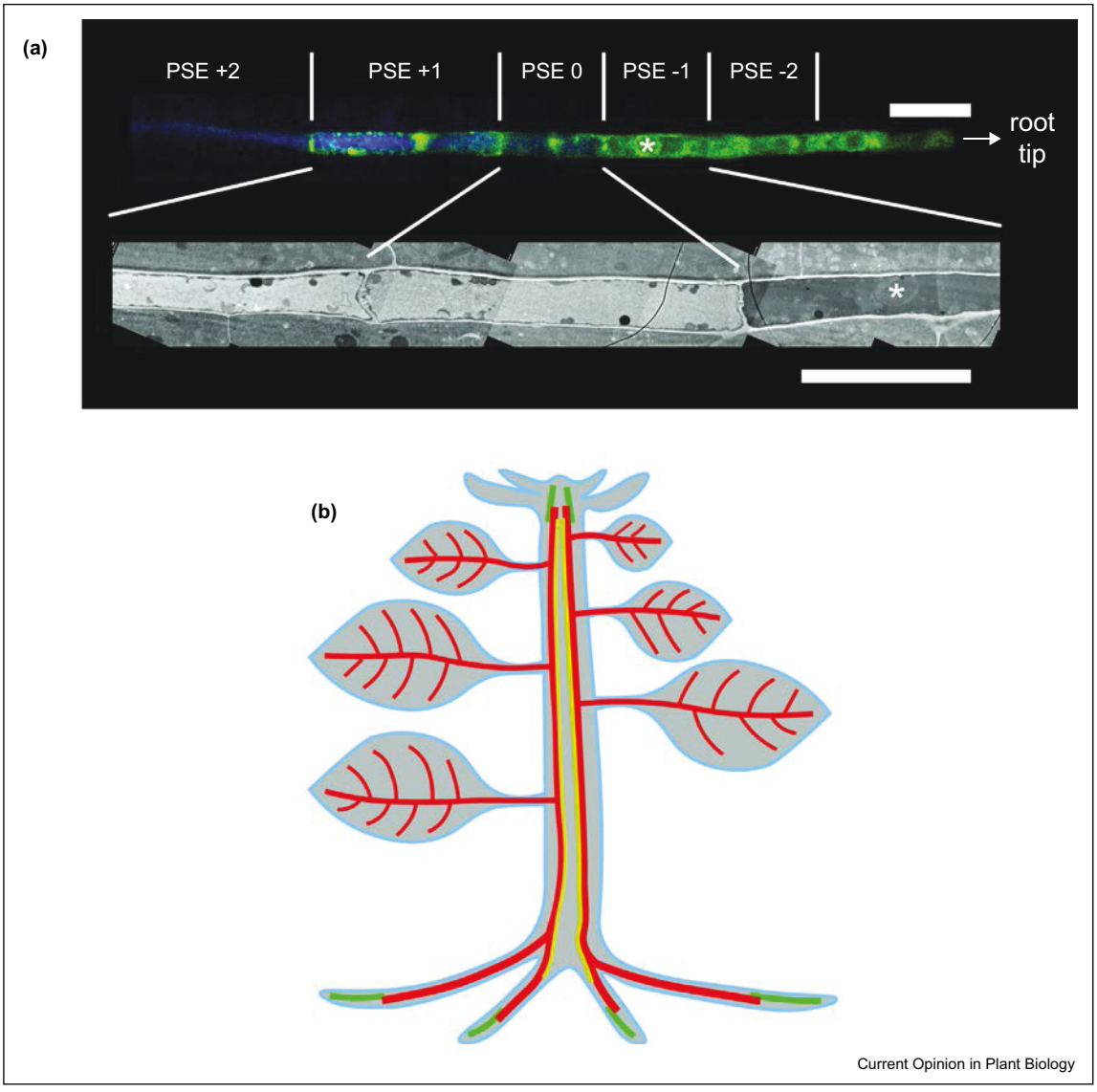
Lack of actin cytoskeleton in mature sieve elements (SEs). **(a)** *In situ* imaging of a transgenic *Arabidopsis* root expressing the actin-binding-domain-protein tagged with cyan fluorescent protein (blue; panel 1). Carboxyfluorescein (yellow; panel 2) loaded into the sieve tube system marks an SE (asterisk) from where it has moved into neighboring companion cells (CCs). Typical actin filaments are visible in CCs but not in the SE (combined image; panel 3). **(b)** Confocal micrograph of transgenic *Nicotiana tabacum* expressing GFP tagged to the ER under the control of the SE-specific SEOR promoter (green). The section was immunolabelled with the anti-actin antibody C4 (red). Actin filaments and tagged ER are seen in the CC and SE, respectively; there is no overlap of the signals. **(c)** Confocal micrograph of a transgenic *N. tabacum* line expressing GFP fused to the actin-binding protein fimbrin (green). Aniline blue stains callose in SEs (blue). Well-developed actin filaments are visible in parenchyma and CCs but not in SEs. Scale bars: *A* = 10 μm; *B* = 20 μm; *C* = 20 μm.

usually characterize actin filaments. The labeled, inhomogeneous structures [11] resembled the parietal ER meshwork reported by others (e.g. Plate 3 in [22]). Therefore, rather than indicating the presence of a functional actin cytoskeleton, the immuno-labeling probably identifies monomeric actin that was translocated in the sieve tubes at the time of fixation. Our interpretation is influenced by our complete failure to detect actin filaments in mature SEs using two fluorescent actin probes, actin-binding-domain-CFP (Figure 1a) and fimbrin-GFP (Figure 1c), both of which labeled actin filaments clearly in CCs. Similarly, we detected actin in CCs but not in SEs by immuno-fluorescence using the same antibody as Hafke *et al.* [11] (Figure 1b). Our data support the conventional wisdom that differentiating SEs dissolve their cytoskeleton. Breakdown products such as monomeric actin may then enter the translocation stream (as suggested in [15]), potentially causing confusion when detected in mature SEs.

Do immature SEs 'contaminate' exudates collected from mature SEs?

Sieve tube exudates can be collected by a variety of methods and are the primary source for analyses of SE contents [23]. The collected sap is subjected to proteomics, metabolomics or RNA analysis. Long-distance signaling by sap

Figure 2

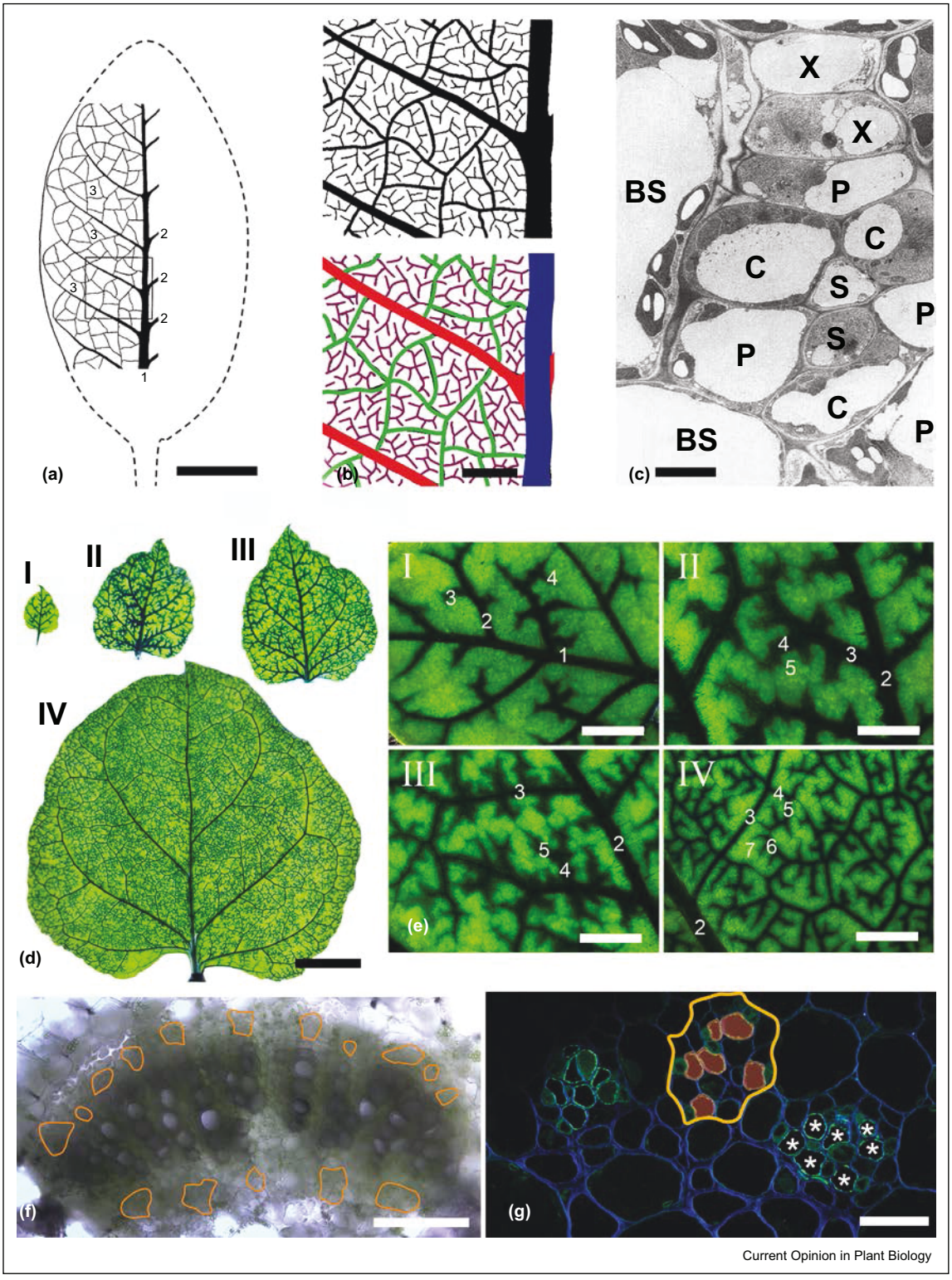


Locations of sieve elements (SEs) potentially contaminating phloem exudates. **(a)** Confocal micrograph (top) showing a terminal file of protophloem sieve elements (PSEs) in the root of a transformed *Arabidopsis*, in which the ER in SEs is tagged with GFP (green). Esculin translocating towards the root apex (i.e. from left to right) appears blue. The most apical SE active in translocation, as indicated by the esculin signal, is PSE 0. Its proximal neighbor, PSE +1, is evidently transporting. Its distal neighbor, PSE −1, still has a nucleus (asterisk) and is not yet connected to the transporting sieve tube. In the electron micrograph (bottom), the cytoplasm appears much denser in PSE −1 than in PSE 0; the degradation of cytoplasmic structures is not completed yet in PSE −1. The degradation products will become part of the sieve tube sap when the sieve pores between PSE 0 and PSE −1 open. At that time, PSE −1 will become the new PSE 0. **(b)** Model showing the location of different SEs and the fate of their cytoplasm following their connection to sieve tubes. With the exception of protophloem SEs (green) whose content will be unloaded directly into the nearest sink, all metaphloem SEs (red) and secondary SEs derived from the cambium (yellow) release their contents into translocating sieve tubes. Scale bars = 10 μm.

components including peptides and RNAs, an important yet controversial topic [24,25•,26•], will be discussed in separate contributions to this issue, but parts of the following discussion may apply to these putative signals.

Up to several thousand proteins have been found in phloem exudates from various species [27], including proteins involved in metabolism, oxidative stress, signaling, protein turnover, as well as structural components of the cytoskeleton, ribosomes, and proteasomes. SEs are connected to neighboring CCs by unique pore-plasmodesmata, and symplasmic import through these channels is thought to explain the presence of the various proteins in exudates. However, the size exclusion limit of the

Figure 3



Anatomical basis for estimating the contribution of differentiating sieve elements (SEs) to the phloem flow exiting a leaf. **(a, b)** Vein system in a *Nicotiana benthamiana* leaf during the source/sink transition. Vein classes defined by branching hierarchy are blue, class 1; red, class 2; green, class 3; purple, class 4 and 5 minor veins (adapted from [31]). **(c)** Electron micrograph of a cross-section of a class 5 vein; S = young SEs, C = companion cells, P = phloem parenchyma cell, BS = bundle sheath cell, x = xylem (a–c slightly modified from [31], Copyright of American Society of Plant Biologists). **(d)** *N. benthamiana* leaves at four stages of development (I–IV), stained with methylene blue to highlight the vein

plasmodesmata between SEs and CCs (70 kDa [26•]) prevents the passage of particles as large as the ribosome and proteasome fragments identified in exudates [28••]. If loading through plasmodesmata from neighboring cells into SEs is impossible, these structures can only originate from within the sieve-tube system itself. This conclusion seems to conflict with the fact that the functional ribosomes required for translation have not been observed in mature SEs yet.

Differentiating SEs could be an alternative source for the macromolecules found routinely in phloem exudates. SE precursor cells contain the standard set of organelles, but at the final stage of differentiation selected cellular structures disintegrate. The degradation products remain within a dense cytoplasmic 'soup' until the sieve pores open (Figure 2a); their subsequent fate depends on the location and nature of the SE (Figure 2b). Protophloem SEs derived from apical meristems are part of the unloading zone of sinks. Their degradation products will not move as they are released at endpoints of phloem transport routes, and ultimately will be driven out of the sieve tube into neighboring cells such as the phloem-pole pericycle [2•]. However, not all new SEs form in sinks (although this sometimes is assumed, e.g. [25•]). Degradation products from secondary phloem SEs in the axial cambium or SEs in source organs such as young leaves [29] will enter the translocation stream. In *Populus tremula* × *alba* leaves, the total SE cross-sectional area increases exponentially from the major veins in the petioles to the minor veins in the blade [30•]. Therefore the proportion of degradation products in the phloem sap exported from a leaf may be significant. Detailed studies on young, still importing *Nicotiana benthamiana* leaves showed that prior to the sink/source transition, minor vein SEs are present but remain immature until the leaf becomes a source [31]. During this transition, the minor vein SEs mature, connect to translocating sieve tubes, and become active in loading [31]. Their intracellular degradation products thus will be found in the phloem sap that exits the leaf.

We evaluated the contribution of young SEs to volume flow based on the data from *N. benthamiana* leaves undergoing the sink/source transition [31]. At that stage, leaves are typically 2.5 cm long and contain five vein classes. The main vein is defined as class 1, and veins branching from it represent class 2. Veins branching from class 2 veins form class 3, and so on. Minor vein sieve elements (class 4 and 5) are present but rest in an immature state until the leaf transitions into a source when the cells mature and become active in loading [31]. The total length of class 4 and 5 minor veins in leaves of this stage is about 1 m (Figure 3a and b). On average, the minor vein SEs shown in [31] have a cross-sectional area of 8.1 μm^2. Since there are two sieve tubes in each minor vein (Figure 3c), a volume of approximately 1.6×10^7 μm^3, or 16 nL, will be released when the young SEs mature. During leaf expansion, new minor veins are added and class 6 and 7 minor veins appear (Figure 3d, e). After 5 days of leaf expansion, the total length of minor veins has increased to roughly 16 m. Thus a total of 256 nL of minor-vein SE contents enter the translocation stream over the 5 days after the leaf has started to export.

How does this quarter microliter of cytoplasmic material compare to the total volume flow of phloem sap from the leaf? All export from the leaf passes through the petiole, which has a main vein with a phloem cross-sectional area of some 32 000 μm^2 (Figure 3f; lateral bundles combined contribute below 10% of the total phloem area and are disregarded here). Since about 25% of this area are covered by SEs (Figure 3g), the estimated conducting area is 8000 μm^2. Flow velocities in petioles of different species vary from 3 to 150 $\mu m/s$ [32]. Lacking data for *Nicotiana*, we assume a velocity of 100 $\mu m/s$, and arrive at an export rate of just below 3 $\mu L/h$. We are considering a leaf over 5 days from the initiation of the sink/source transition, a stage of leaf growth often used for exudate collection. At the beginning of this period, phloem export velocity is zero, increasing over time as the exporting leaf grows. Thus, in our example, significantly less than 360 μL phloem sap is exported within 5 days, of which some 0.25 μL are derived from differentiating SEs. In other words, the cytoplasm of differentiating SEs represents significantly more than 0.07% of the phloem sap passing through the petiole during this period. This proportion, and the corresponding dilution factor of about 1400, refers to fluid volume *per se*. The proportion of young SE-derived material in a given class of molecules in the phloem sap might be different, depending on any active loading or degradation that may occur. Measurements of the proportion of RNA in sieve tube sap range from 0.003% of the fresh weight in a recent study [33] down to <0.0002% in a classical investigation [34]. Conventional text-book wisdom suggests that RNA represents over 1% of the fresh-weight of eukaryotic cells [35]. Assuming these figures hold in our example, and applying

(Figure 3 Legend Continued) system. All pictures are taken at the same magnification. It is apparent that the number of vein orders increases with increasing area of the lamina. **(e)** Higher magnification of the same leaves as in (d); 1–7 indicate vein classes. **(f)** Cross-section of the central vein in the petiole of leaf IV in (d). Orange outlines mark the area of internal and external phloem in the bicollateral bundles interrupted by rays for parenchyma cells. **(g)** Confocal micrograph of a petiole cross-section in a transgenic *N. tabacum* line carrying GFP tagged to the ER in sieve tubes (asterisks). In one bundle, the phloem is outlined in orange and SEs are marked red. About 25% of the phloem area are covered by sieve elements. Individual phloem areas are interrupted by large parenchyma cells. Scale bars: *A* = 5 mm; *B* = 1 mm; *C* = 4 μm; *D* = 2 cm; *E* = 2 mm; *F* = 200 μm; *G* = 30 μm.

the volumetric dilution factor calculated above, we find that RNA released from young SEs could account for anywhere between one quarter and the three-fold of the RNA in the phloem stream that exits the leaf. Similar arguments can be made for SEs differentiating in the axial cambium (Figure 3), which release the remnants of their cytoplasm into the translocation stream when they connect to it. Obviously, our arguments call for experimental verification. However, we suggest that the cellular components of immature SEs might contribute significantly to the composition of phloem exudates.

Waste not, want not

The release of degradation products from differentiating SEs into the phloem sap, instead of recycling them in source tissues, might be beneficial. As recently shown for growing *Arabidopsis* root tips, large molecules are unloaded from protophloem SEs into the phloem pole pericycle cells through specific funnel plasmodesmata with large size exclusion limits [2•]. Delivery of the degradation products to sinks such as root growth zones could provide carbon and nitrogen skeletons for growth and development.

Conclusions

The mere presence of a molecule in phloem sap is insufficient evidence for a specific function of that molecule within SEs, or indeed the sink to which it is delivered [36]; additional functional evidence is imperative. For example, blue native electrophoresis applied to phloem exudates indicated the presence of ribosomal and proteasomal protein complexes [28••]. However, essential functional components of ribosomes were missing; translation in mature SEs therefore seems unlikely. In contrast, functional tests demonstrated proteasome activity [28••], providing strong support for the hypothesis that proteasomes in sieve tubes function in pathogen defense [37]. It will be interesting to see whether these proteasomes originate from differentiating SEs.

Volume flow in the phloem of a young exporting leaf will increase with the expansion of photosynthetic area. The release of cytoplasmic materials from differentiating SE, on the other hand, will slow as the leaf approaches full expansion. Thus, using fully expanded leaves for exudate studies would minimize contamination from immature SEs. However, secondary growth in axial cambia will continue to release cytoplasmic SE contents into the system. Due attention to these developmental and structural aspects will improve the rigor of exudate analyses.

Acknowledgements

We thank Elison B. Blancaflor for providing seeds of transgenic *Arabidopsis* lines with the actin binding domain-CFP reporter. This work was supported by the National Science Foundation [NSF-IOS 1656769, 1456682] (MK) and the Biotechnology and Biological Sciences Research Council [BB/M025160/1] (KJO).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Heo J-O, Blob B, Helariutta Y: **Differentiation of conductive cells: a matter of life and death**. *Curr Opin Plant Biol* 2017, **35**:23-29.

2. • Ross-Elliott TJ, Jensen KH, Haaning KS, Wagner BM, Knoblauch J, Howell AH, Mullendore DL, Monteith AG, Paultre D, Yan DW *et al.*: **Phloem unloading in *Arabidopsis* roots is convective and regulated by the phloem pole pericycle**. *eLife* 2017, **6**:e24125.

This paper shows how unloading takes place in the protophloem of *Arabidopsis* roots. A new type of plasmodesmata of high conductivity that enable a combination of bulk flow and diffusion is described.

3. Knoblauch M, Stubenrauch M, Van Bel AJE, Peters WS: **Forisome performance in artificial sieve tubes**. *Plant Cell Environ* 2012, **35**:1419-1427.

4. Knoblauch M, Froelich DR, Pickard WF, Peters WS: **SEORious business: structural proteins in sieve tubes and their involvement in sieve element occlusion**. *J Exp Bot* 2014, **65**:1879-1893.

5. Mullendore DL, Windt CW, Van As H, Knoblauch M: **Sieve tube geometry in relation to phloem flow**. *Plant Cell* 2010, **22**:579-593.

6. Fitzgibbon J, Bell K, King E, Oparka K: **Super-resolution imaging of plasmodesmata using three-dimensional structured illumination microscopy**. *Plant Physiol* 2010, **153**:1453-1463.

7. Knoblauch M, Oparka K: **The structure of the phloem — still more questions than answers**. *Plant J* 2012, **70**:147-156.

8. Ehlers K, Knoblauch M, van Bel AJE: **Ultrastructural features of well-preserved and injured sieve elements: minute clamps keep the phloem transport conduits free for mass flow**. *Protoplasma* 2000, **214**:80-92.

9. Froelich DR, Mullendore DL, Jensen KH, Ross-Elliott TJ, Anstead JA, Thompson GA, Pelissier HC, Knoblauch M: **Phloem ultrastructure and pressure flow: Sieve-Element-Occlusion-Related agglomerations do not affect translocation**. *Plant Cell* 2011, **23**:4428-4445.

10. Behnke HD, Sjolund RD: *Sieve Elements — Comparative Structure, Induction and Development*. Springer; 1990.

11. Hafke JB, Ehlers K, Foller J, Holl SR, Becker S, van Bel AJE: **Involvement of the sieve element cytoskeleton in electrical responses to cold shocks**. *Plant Physiol* 2013, **162**:707-719.

12. van Bel AJE, Furch ACU, Will T, Buxa SV, Musetti R, Hafke JB: **Spread the news: systemic dissemination and local impact of Ca^{2+} signals along the phloem pathway**. *J Exp Bot* 2014, **65**:1761-1787.

13. Lin MK, Lee YJ, Lough TJ, Phinney BS, Lucas WJ: **Analysis of the pumpkin phloem proteome provides insights into Angiosperm sieve tube function**. *Mol Cell Proteomics* 2009, **8**:343-356.

14. Schobert C, Baker L, Szederkényi J, Großmann P, Komor E, Hayashi H, Chino M, Lucas WJ: **Identification of immunologically related proteins in sieve-tube exudate collected from monocotyledonous and dicotyledonous plants**. *Planta* 1998, **206**:245-252.

15. Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J: **Towards the proteome of *Brassica napus* phloem sap**. *Proteomics* 2006, **6**:896-909.

16. Fröhlich A, Gaupels F, Sarioglu H, Holzmeister C, Spannagl M, Durner J, Lindermayr C: **Looking deep inside: detection of low-abundance proteins in leaf extracts of *Arabidopsis* and phloem exudates of pumpkin**. *Plant Physiol* 2012, **159**:902-914.

17. Batailler B, Lemaitre T, Vilaine F, Sanchez C, Renard D, Cayla T, Beneteau J, Dinant S: **Soluble and filamentous proteins in**

***Arabidopsis* sieve elements**. *Plant Cell Environ* 2012, **35**:1258-1273.

18. Anstead JA, Hartson SD, Thompson GA: **The broccoli (*Brassica oleracea*) phloem tissue proteome**. *BMC Genomics* 2013, **14**:764.

19. Buxa SV, Degola F, Polizzotto R, De Marco F, Loschi A, Kogel K-H, di Toppi LS, van Bel AJE, Musetti R: **Phytoplasma infection in tomato is associated with re-organization of plasma membrane, ER stacks, and actin filaments in sieve elements**. *Front Plant Sci* 2015, **6**:650.

20. Furch ACU, Buxa SV, van Bel AJE: **Similar intracellular location and stimulus reactivity, but differential mobility of tailless (*Vicia faba*) and tailed forisomes (*Phaseolus vulgaris*) in intact sieve tubes**. *PLOS ONE* 2015, **10**:0143920.

21. Anstead JA, Froelich DR, Knoblauch M, Thompson GA: ***Arabidopsis* P-protein filament formation requires both AtSEOR1 and AtSEOR2**. *Plant Cell Physiol* 2012, **53**:1033-1042.

22. Esau K: **Developmental features of the primary phloem in *Phaseolus vulgaris* L.**. *Ann Bot* 1978, **42**:1-13.

23. Dinant S, Kehr J: **Sampling and analysis of phloem sap**. In *Plant Mineral Nutrients: Methods and Protocols.* Edited by Maathuis FJM. Humana Press; 2013:185-194.

24. Ham B-K, Lucas WJ: **Phloem-mobile RNAs as systemic signaling agents**. *Annu Rev Plant Biol* 2017, **68**:173-195.

25. • Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang WN, Yang L, Minambres M, Walther D, Schulze WX, Praz-Ares J *et al.*: **Endogenous Arabidopsis messenger RNAs transported to distant tissues**. *Nat Plants* 2015, **1**:15025.

Studying grafts of genetically different *Arabidopsis* ecotypes, the authors demonstrate the unexpected phenomenon of bidirectional RNA transport.

26. • Paultre DSG, Gustin MP, Molnar A, Oparka KJ: **Lost in transit: long-distance trafficking and phloem unloading of protein signals in Arabidopsis homografts**. *Plant Cell* 2016, **28**:2016-2025.

Using a combination of imaging and bioinformatics, this work shows that for proteins synthesised in companion cells, the size exclusion limit for symplastic transfer into the sieve element is about 70 kDa.

27. Turnbull CGN, Lopez-Cobollo RM: **Heavy traffic in the fast lane: long-distance signalling by macromolecules**. *New Phytol* 2013, **198**:33-51.

28. •• Ostendorp A, Pahlow S, Krüßel L, Hanhart P, Garbe MY, Deke J, Giavalisco P, Kehr J: **Functional analysis of Brassica napus phloem protein and ribonucleoprotein complexes**. *New Phytol* 2017, **214**:1188-1197.

This study analyzes native protein complexes in the phloem sap of *Brassica napus.* Most importantly, the authors evaluate the activity of the complexes in mature sieve tubes.

29. Turgeon R: **Phloem loading: how leaves gain their independence**. *BioScience* 2006, **56**:15-24.

30. • Carvalho MR, Turgeon R, Owens T, Niklas KJ: **The scaling of the hydraulic architecture in poplar leaves**. *New Phytol* 2017, **214**:145-157.

An important step towards a fully quantitative understanding of vascular structure on the organ level.

31. Roberts AG, Cruz SS, Roberts IM, Prior D, Turgeon R, Oparka KJ: **Phloem unloading in sink leaves of *Nicotiana benthamiana*: comparison of a fluorescent solute with a fluorescent virus**. *Plant Cell* 1997, **9**:1381-1396.

32. Jensen KH, Lee J, Bohr T, Bruus H, Holbrook NM, Zwieniecki MA: **Optimality of the Münch mechanism for translocation of sugars in plants**. *J Roy Soc Interface* 2011, **8**:1155-1165.

33. Buhtz A, Springer F, Chappell L, Baulcombe DC, Kehr J: **Identification and characterization of small RNAs from the phloem of *Brassica napus***. *Plant J* 2008, **53**:739-749.

34. Ziegler H, Kluge M: **Die Nucleinsäuren und ihre Bausteine im Siebröhrensaft von *Robinia pseudacacia***. *Planta* 1975, **58**:144-153.

35. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P: *Molecular Biology of the Cell*. edn 4. Garland Science; 2002.

36. Oparka KJ, Santa Cruz S: **The great escape: phloem transport and unloading of macromolecules**. *Annu Rev Plant Physiol Plant Mol Biol* 2000, **51**:323-347.

37. Hipper C, Brault V, Ziegler-Graff V, Revers F: **Viral and cellular factors involved in phloem transport of plant viruses**. *Front Plant Sci* 2013, **4**:154.