

Diversity of funnel plasmodesmata in angiosperms: the impact of geometry on plasmodesmal resistance

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9 **Abstract**

10 In most plant tissues, threads of cytoplasm, or plasmodesmata, connect the protoplasts via pores
11 in the cell walls. This enables symplasmic transport, for instance in phloem loading, transport,
12 and unloading. Importantly, the geometry of the wall pore limits the size of the particles that may
13 be transported, and also (co-)defines plasmodesmal resistance to diffusion and convective flow.
14 However, quantitative information on transport through plasmodesmata in non-cylindrical cell
15 wall pores is scarce. We have found conical, funnel-shaped cell wall pores in the phloem-
16 unloading zone in growing root tips of five eudicot and two monocot species, specifically
17 between protophloem sieve elements and phloem pole pericycle cells. 3D reconstructions by
18 electron tomography suggested that funnel plasmodesmata possess a desmotubule but lack
19 tethers to fix it in a central position. Model calculations showed that both diffusive and hydraulic
20 resistance decrease drastically in conical cell wall pores compared to cylindrical channels, even
21 at very small opening angles. Notably, the effect on hydraulic resistance was relatively larger.
22 We conclude that funnel plasmodesmata generally are present in specific cell-cell interfaces in
23 angiosperm roots, where they appear to facilitate symplasmic phloem unloading. Interestingly,
24 cytosolic sleeves of most plasmodesmata reported in the literature do not resemble straight
25 annuli but possess variously shaped widenings. Our evaluations suggest that widenings too small
26 for identification on electron micrographs may drastically reduce the hydraulic and diffusional
27 resistance of these pores. Consequently, theoretical models assuming cylindrical symmetries will
28 underestimate plasmodesmal conductivities.

29 **Introduction**

30 Cells in plant tissues are defined by cell walls, rigid extracellular networks consisting of
31 polysaccharides and, to a lesser degree, proteins. The cells are not physically isolated, though, as
32 they are connected by thin cytoplasmic threads that extend through pores in the walls. The size
33 and structure of these plasmodesmata can be modified by the living cells depending on their
34 physiological and developmental requirements (Peters et al. 2021). Plasmodesmata may be
35 simple (i.e., cylindrical), branched, or of more complex, often asymmetric geometries (Lee and
36 Frank 2018). The interfaces between certain cell types may be characterized by specific
37 plasmodesmal structures. For instance, the walls forming the interface between the sieve
38 elements and companion cells in the phloem are perforated by so-called pore-plasmodesma units,
39 branching cell wall pores with a large opening on the side of the sieve element and several
40 smaller openings facing the companion cell (Esau and Thorsch 1985).

41 Plasmodesmata enable diffusion and in some cases bulk flow between cells. Such movement
42 can be visualized using fluorescent reporter techniques including fluorochrome microinjection
43 (Goodwin et al. 1990; Barton et al. 2011), fluorescence recovery after photobleaching (Wang et
44 al. 2020; Rutschow et al. 2011), photoactivatable fluorochromes (Liesche and Schulz 2012), and
45 photo-inducible fluorescent proteins (Gerlitz et al. 2018). Obviously, the size of particles that
46 may move through a plasmodesma is limited by the dimensions of the plasmodesma;
47 plasmodesmata function as sieves, as it were (Schulz 1999). Size exclusion limits below 1 kDa
48 have been reported from tissues in photoassimilate-exporting leaves that often possess branched
49 plasmodesmata (Oparka et al. 1999). Simple plasmodesmata seem to be involved in a specific
50 assimilate export mechanism known as polymer trap, which requires size exclusion limits around
51 0.5 kDa (Comtet et al. 2017). In contrast, larger molecules of up to 40 kDa appear to move

52 through simple plasmodesmata in photoassimilate-importing tissues (Opalka et al. 1999; Nicolas
53 et al. 2017; Lee and Frank 2018), while pore-plasmodesma units enable the exchange of probes
54 of up to 70 kDa (Opalka and Turgeon 1999; Fitzgibbon et al. 2013). Funnel-shaped
55 plasmodesmata in the root unloading zone of *Arabidopsis* permit movements of molecules of at
56 least 112 kDa (Ross-Elliott et al. 2017).

57 The developmental and cell type-specific variation of plasmodesmal size exclusion limits
58 indicates active regulation and thus physiological significance of the effective size of the
59 plasmodesmal pore. Therefore the geometry of plasmodesmata must be expected to play a role in
60 controlling cell-to-cell conductivity. Most theoretical plasmodesma models for quantitative
61 evaluations generally assumed coaxial symmetry with a straight, cylindrical cell wall pore, based
62 on interpretations of electron micrographs by e.g. Ding et al. (1992) and Waigmann et al. (1997).
63 Consequently, models including a cytosolic sleeve mostly assumed this sleeve to be tubular with
64 constant radius and annular cross-sectional shape, or to consist of a group of circularly arranged
65 cylindrical tubes (Comtet et al. 2017; Liesche and Schulz 2013; Park et al. 2019). In reality,
66 however, asymmetric and irregular shapes are common. For example, central cavities – diameter
67 widenings in the center of plasmodesmata – have been described repeatedly (Ding et al. 1992;
68 Nicolas et al. 2017; Fitzgibbon et al. 2010), but rarely were considered in theoretical analyses of
69 plasmodesmal transport. Blake (1978) modeled convective flow but not diffusion in
70 plasmodesmata that widened in the center, while Deinum et al. (2019) analyzed diffusion but not
71 convective flow. Electrical effects have never been evaluated, although increasing plasmodesmal
72 diameter at constant Debye length will reduce the influence of static wall charges on the
73 movement of charged particles in the cytosol (Peters et al. 2021).

74 Root growth is fueled by materials that are unloaded from protophloem sieve elements
75 (PSEs), the youngest fully functional components of the sieve tubes that reach the root
76 elongation zone. There are two such tubes in *Arabidopsis*, and the unloading sieve elements
77 typically have five neighbors: two companion cells, an immature metaphloem sieve element, and
78 two phloem pole pericycle cells (PPPs). The PSE/PPP interfaces are characterized by unusual,
79 funnel-shaped plasmodesmata (Ross-Elliott et al. 2017); apparently analogous plasmodesmata in
80 *Hordeum vulgare* had been briefly discussed by Warmbrodt (1985). Initial calculations indicated
81 that the conical funnel shape facilitated convective phloem unloading at low pressure
82 differentials (Ross-Elliott et al. 2017). These findings supported the idea that the PSE/PPP
83 interface has a specific significance for phloem unloading in *Arabidopsis* roots.

84 Since phloem unloading mechanisms are of major importance in the context of food
85 production for human and livestock consumption, detailed knowledge of the distribution of
86 funnel plasmodesmata in species other than *Arabidopsis* as well as a better understanding of the
87 physics of transport through non-cylindrical plasmodesmata would seem desirable. Therefore,
88 and because funnel plasmodesmata appeared a convenient case for studying the impact of non-
89 cylindrical pore shapes on plasmodesmal transport, we first established the general occurrence of
90 funnel plasmodesmata in angiosperms. Then, we generated 3D reconstructions based on electron
91 tomograms of these plasmodesmata to evaluate flow patterns and resistances, and modeled
92 physical flow characteristics in idealized cylindrical and conical plasmodesmata, to evaluate the
93 effects of various pore geometries. As a general conclusion, we suggest that often overlooked
94 deviations from a straight, cylindrical shape can reduce the plasmodesmal resistance to bulk flow
95 and diffusion significantly.

96

Results

97

Funnel plasmodesmata in seven species: basic observations

98

To investigate the ultrastructure of the interface of the protophloem with surrounding cells, we
99 adapted microwave-supported fixation protocols for five eudicots from various families and two
100 Poaceae species as representatives of the monocots. Variations in root thickness, tissue density,
101 and other structural parameters made it necessary to adjust protocols for several species (see
102 Methods). We note that protophloem sieve elements in roots of most species are more difficult to
103 preserve for electron microscopy than those in the thin roots of *Arabidopsis*. Vitrification by
104 cryo-fixation usually fails as the protophloem is located too deep within the organ to achieve the
105 required freezing speeds.

106

With few exceptions, eudicots exhibit a bi-, tri-, tetra-, or pentarch architecture of the
107 primary root, i.e. the roots possess two, three, four, or five protoxylem/protophloem units. In
108 contrast, Poaceae roots generally are polyarch, showing a greater number of protophloem
109 strands. Our study species confirmed this pattern (Fig. 1A, tetrarch root of *Ipomoea nil*; Fig. 1B,
110 polyarch root of *Triticum aestivum*). Viewed on cross-sections of the zone of phloem unloading,
111 the protophloem sieve elements (PSEs) of the eudicots examined were connected to five cells
112 (Fig. 1C). These comprised two phloem pole pericycle cells (PPPs), two companion cells (CCs),
113 and one immature metaphloem sieve element (MP). In contrast, protophloem sieve elements had
114 only four direct neighbors in the Poaceae examined, as they were not reached by metaphloem
115 sieve elements in the phloem unloading zone (Fig. 1D).

116

We found funnel-shaped plasmodesmata in all species examined (Fig. 1E–K). As in
117 *Arabidopsis*, the occurrence of these funnel plasmodesmata was restricted to the walls between
118 protophloem sieve elements and phloem pole pericycle cells, the PSE/PPP interfaces. The

119 surfaces of these walls appeared relatively smooth in the eudicots (Fig 1E–I). In the Poaceae, the
120 wall surfaces on the side of the sieve elements were rough with numerous protrusions and
121 invaginations (Fig. 1J, K). In all species, the wider apertures of funnel plasmodesmata were
122 always on the side of the protophloem sieve element (Fig. 1E–K), and thus served as inlet
123 apertures of the plasmodesmata in phloem unloading.

124 **Geometry of funnel-shaped cell wall pores**

125 In general, the geometries of the funnel-shaped cell wall pores were somewhat irregular due to
126 their rugged inner surfaces. Nonetheless, the diameter, D_{PSE} , of the wider plasmodesmal aperture
127 on the side of the protophloem sieve element as well as the length, L , of the plasmodesma
128 (equivalent to cell wall thickness) could be determined on numerous micrographs ($n = 18$ for *S.*
129 *scutellarioides*, $n = 30$ for all other species; examples are shown in Fig. 1E–K, and data are
130 compiled in Supplemental Table S1). If the opening angles, θ , of the funnel-shaped cell wall
131 pores were constant, D_{PSE} should increase with increasing L for geometrical reasons (Fig. 2A).
132 However, there was no correlation between the measured values of D_{PSE} and L (Fig. 2B),
133 suggesting that θ should decrease with L . The estimation of θ was difficult as the narrow
134 aperture diameters facing the phloem pole pericycle cells, D_{PPP} , could not be determined
135 unequivocally on many of the micrographs. Because our rough estimates of D_{PPP} on all analyzed
136 micrographs ranged between 17 and 51 nm, we calculated θ for each plasmodesma with D_{PPP} set
137 to 20 and 50 nm, to cover the range of realistically expectable values. In fact, θ showed a tendency
138 to decline with increasing wall thickness as indicated by the Geometric Mean Functional
139 Relationship (GMFR) between the two parameters (Fig. 2C), but this relationship was far too
140 weak to support general conclusions ($r^2 = 0.12$ for data calculated with $D_{PPP} = 20$ nm, and $r^2 =$

141 0.08 for $D_{PPP} = 50$ nm). While θ varied widely, there were species-specific trends; the median of
142 θ determined separately for each species was smallest in *C. speciosa* and five to ten times higher
143 in *O. sativa*, depending on which value of D_{PPP} was assumed (Fig. 2D).

144 **3D reconstructions of individual plasmodesmata**

145 Our above considerations of the geometries of funnel-shaped cell wall pores were based on the
146 simplifying assumptions presented in Fig. 2A. To obtain a more realistic picture of the structure
147 of individual funnel plasmodesmata, we generated 3D reconstructions by electron tomography.
148 We acquired 220 nm slices of stained, resin-embedded samples for all species except *O. sativa*,
149 which required 280 nm sections, wide enough to carry complete funnel plasmodesmata. Our
150 reconstructions showed funnel plasmodesmata of varying, irregular shapes (Fig. 3). While we
151 were able to identify desmotubules, it must be cautioned that the apparent diameters of these
152 structures depend on parameters including sample thickness, resin hardness, desmotubule
153 location, staining time, etc. In none of our image series, desmotubules appeared as sharply
154 bordered structures but rather showed as gradients of contrast intensity. Therefore their exact
155 dimensions were not always clearly discernible, and variations in apparent desmotubule diameter
156 might reflect methodological uncertainty as much as natural variability of the actual structure.
157 Given this caveat, it still appears noteworthy that funnel plasmodesmata showed no signs of
158 tether-like connections between desmotubule and plasma membrane. Rather than being fixed in
159 the center of the pore, the location of desmotubules in our reconstructions was highly variable
160 (Fig. 3).

161 In the next step, we used electron tomography surface reconstructions of selected funnel
162 plasmodesmata as templates and extracted tetrahedral grids for theoretical hydrodynamics

163 analyses by finite element methods (Bassi and Rebay 1997). Flow simulations confirmed that
164 flow velocity under a given cell-to-cell gradient of hydrostatic pressure increased with
165 decreasing diameter of the funnel plasmodesmata (Fig. 4). Since the flow rate in a channel equals
166 the pressure gradient divided by hydrodynamic resistance, this implied that overall flow
167 resistance in funnel plasmodesmata is mostly determined by the width of their narrow outlet
168 apertures into the phloem pole pericycle cells.

169 **Theoretical analysis of funnel geometry effects on diffusion and bulk flow**

170 Since the development of a realistic model describing rates of diffusion and of convective flow
171 as well as electrokinetic effects was beyond the scope of the present study, we restricted further
172 theoretical analysis of the impact of funnel geometries on plasmodesmal transport to the
173 resistances offered by various channel geometries to bulk flow and diffusion. As our standard or
174 control condition, we considered cylindrical channels of radius a and length L (Fig. 5A, left). A
175 rod of radius b in the center of the channels mimicked a desmotubule, and flow and diffusion
176 were assumed to occur only in the annular sleeve between the wall of the channel and the central
177 rod (Fig. 5A, left). In our calculations, we set channel length L , equivalent to wall thickness, to
178 400 nm. While radius b was kept constant at 7.5 nm, the outer radius, $a = b + s$, was varied to
179 obtain sleeve widths (s) of 2, 4, and 8 nm (the latter value may seem high, but proteins of 112
180 kDa, corresponding to a hydrodynamic diameter of ~8 nm, may pass through funnel
181 plasmodesmata; Ross-Elliott et al. 2017). As a result, we had three cylindrical channel models
182 that differed only in radius a and thus in sleeve width. These models served as cylindrical
183 standards to which conical channels – i.e., funnel-shaped ones – could be compared. In these
184 conical channels, radius b remained constant but radius a increased steadily from the smaller, or
185 outlet aperture, toward the larger, or inlet aperture (Fig. 5A, right). Consequently, sleeve width s

186 and the cross-sectional sleeve area A increased in the same direction as well, in dependence on
187 the magnitude of θ , the angle between the channel wall and the surface of the central rod (Fig.
188 5A, right). Sleeve widths at the outlet apertures – minimum sleeve widths, in other words – were
189 set to 2, 4, and 8 nm, to allow for direct comparison to the cylindrical channels defined above.

190 The hydraulic resistances of conical channels decreased drastically even with very small
191 angles θ (Fig. 5B). On the other hand, the resistance offered by channels with wider sleeves was
192 substantially lower than that found in narrower ones for all values of θ (Fig. 5B). However, the
193 overlap in the vertical direction of the curves in Fig. 5B indicated that conical geometries in
194 channels with narrow sleeves could reduce the hydraulic resistance to values found in cylindrical
195 channels with much wider sleeves. For instance, the resistance of a cylindrical channel with an 8
196 nm sleeve was met by conical channels with 4 and 2 nm minimum sleeve widths when θ reached
197 2.4° and 11° , respectively (highlighted in Fig. 5B). Values of diffusive resistance did not differ
198 as strongly between channels of different sleeve widths for any given angle θ (Fig. 5C) as
199 hydraulic resistance did (Fig. 5B). Presumably, this qualitative difference stems from the fact
200 that diffusive resistance is inversely proportional to the conductive area, whereas hydraulic
201 resistance scales with the inverse area squared (eqs. 8 and 11). As a consequence, conical
202 channels of 4 and 2 nm minimum sleeve width showed the same diffusive resistance as a
203 cylindrical channel with an 8 nm sleeve at angles θ of only 1.5° and 2.8° , respectively (Fig. 5C).

204 When we normalized the reductions of hydraulic resistance computed for conical channels
205 with respect to the resistances of cylindrical channels of the same sleeve widths, it became clear
206 that the effects were relatively larger in channels with narrower sleeves. For example, hydraulic
207 resistance was reduced to 10% of that found in cylindrical channels of 2, 4, and 8 nm sleeve
208 width in conical channels with angles θ of 1.3° , 2.5° , and 4.6° , respectively, as highlighted in

209 Fig. 6A. The effects of conical geometries on diffusive resistance were qualitatively similar but
210 relatively less pronounced. Reductions of resistance to 10% required angles θ of 5.9°, 9.8°, and
211 16° in channels of 2, 4, and 8 nm minimum sleeve width, respectively (Fig. 6B). Plots of the
212 ratios of the modulations of hydraulic and diffusive resistances computed for conical model
213 channels (Fig. 7) suggested that relative reductions of hydraulic resistance across the range of
214 cell wall pore opening angles observed in real cells (Fig. 2) were two- to five-fold larger than the
215 corresponding relative changes in diffusive resistance. As a result, convective flow will become
216 more important relative to diffusion when θ increases.

217 **Discussion**

218 Most structural and functional plasmodesma studies have been conducted on accessible tissues
219 such as trichomes (Oparka and Prior 1992; Christensen et al. 2009; Barton et al. 2011; Howell et
220 al. 2020), the leaf epidermis (Fitzgibbon et al. 2013), and the calyptra and root meristem (Nicolas
221 et al. 2017). Unfortunately, some of the physiologically most important cases of plasmodesmal
222 transport occur in less accessible tissues, for example the terminal sieve elements of the sieve
223 tubes that deliver the fuel for root growth. These cells are deeply embedded in the central
224 cylinder, which itself is covered by multiple cell layers of the root cortex. We modified fixation
225 techniques for electron microscopy that previously had been applied successfully in *Arabidopsis*,
226 and found funnel plasmodesmata in the phloem unloading zone in root tips of all seven
227 angiosperms examined (Fig. 1).

228 The geometry of funnel plasmodesmata seemed to differ between species. Estimated
229 opening angles of the cell wall pores clustered around 2°–5° in *C. speciosa* and 15°–22° in *O.
230 sativa*; the other five species showed intermediate values (Fig. 2D). Such structural variability

231 does not necessarily imply significant functional differences. Our theoretical evaluations
232 indicated that small opening angles have comparatively large effects on the hydraulic and
233 diffusive resistance of model channels designed to resemble funnel plasmodesmata structurally
234 (Figs. 5, 6). Compared to cylindrical, simple plasmodesmata, and assuming a width of the
235 cytosolic sleeve of 4 nm, opening angles as found in *C. speciosa* (around 3°) would be expected
236 to reduce the hydraulic resistance by over 90%, and the diffusive resistance by some 70% (Fig.
237 6A,B). The much larger wall pore opening angles measured in *O. sativa* (around 19°) produce
238 only modestly increased effects, reducing hydraulic resistance by about 98% and diffusive
239 resistance by 94%. Evidently, the physical resistance to symplasmic phloem unloading by bulk
240 flow and diffusion is significantly decreased by the funnel-like shape of some of the
241 plasmodesmata involved in all of the species studied. We emphasize that at standard phloem
242 flow velocities, the entire volume of a sieve element is exchanged within one or a few seconds
243 (Froelich et al. 2011); the rates at which sieve tubes are unloaded obviously have to be
244 commensurate. The specific occurrence of funnel plasmodesmata in the interfaces between
245 protophloem sieve elements and phloem pole pericycle cells supports the idea that these cells
246 provide the main symplasmic route for the required high-capacity phloem unloading.

247 In this context it seems of interest that for values of θ that correspond to the opening angles
248 estimated for real cells (Fig. 2C), the theoretical reduction in relative hydraulic resistance is two
249 to five times larger than the reduction of diffusive resistance (Fig. 6C). Consequently, the
250 balance between convective and diffusive processes in overall symplasmic transport is expected
251 to shift toward bulk movements when funnel plasmodesmata are formed between cells. This
252 supports the view that rapid phloem unloading in root tips proceeds mainly as bulk flow.

253 Rather than building structurally complex cytoplasmic bridges such as funnel
254 plasmodesmata, plant cells could form larger numbers of simple plasmodesmata per unit cell
255 wall area or widen the diameters of existing simple plasmodesmata to increase symplasmic
256 transport capacity. We see at least two functional factors that might have favored the evolution of
257 funnel plasmodesmata.

258 First, many sink organs including root tips are actively growing, which requires complex
259 fine-tuning of the mechanical properties of the expanding cell walls (Cosgrove 2018).
260 Conceivably, increased densities and/or diameters of the pores in expanding cell walls could
261 interfere with the mechanical control of the growth process. Funnel-shaped cell wall pores
262 therefore may represent a compromise between the requirements for rapid symplasmic transport
263 on one hand and the maintenance of the mechanical integrity of the growing cell wall on the
264 other.

265 Second, as discussed in the Introduction, plasmodesmata function like sieves, preventing
266 particles above a critical size from passing through the narrow cytosolic sleeves. In contrast to
267 cylindrical pores with expanded diameters, funnel plasmodesmata allow for enhanced transport
268 rates while retaining their cargo size-based selectivity. Therefore the presence of funnel
269 plasmodesmata in root tips is in line with the assumption of a physiological necessity for
270 controlling the size of particles that leave the sieve tube. Unusually large molecules in the sieve
271 tube stream include ribosomal subunits (Ostendorp et al. 2017) and other cytoplasmic
272 degradation products from developing sieve elements (Knoblauch et al. 2018). The hypothesis
273 that funnel plasmodesmata control the efflux of these and other large molecules from sieve tubes
274 is supported by observations made in *Arabidopsis* roots. There, small molecules moved from
275 sieve elements to phloem pole pericycle cells continuously whereas large proteins entered the

276 latter in a pulsed manner named ‘batch unloading’ (Ross-Elliott et al. 2017). Evidently, the
277 movement of various molecules through funnel plasmodesmata was differentially regulated
278 depending on molecule size. How batch unloading works remains unclear at this time, but
279 thermal motion of the desmotubule could provide a simple but sufficient explanation, according
280 to the hypothetical cargo-gating mechanism (Peters et al. 2021). A tight control of sieve tube
281 efflux based on molecule size might confer advantages to plants battling viruses that utilize the
282 sieve tubes as routes for systemic infection. Generally, virus particles are too large to pass
283 through plasmodesmata, and require specific movement proteins encoded in the viral genome to
284 enter sieve elements (Nelson 2005). In contrast to the mechanisms by which viruses enter sieve
285 tubes, their exit mechanism(s) is mostly unknown (Hipper et al. 2013). Notably, there is no
286 protein synthesizing machinery in sieve elements that a virus could hijack to produce support
287 proteins. By retaining one comparatively narrow aperture, funnel plasmodesmata might at least
288 slow the systemic spread of viruses while massively increasing their conductivity for smaller
289 particles.

290 Finally, we emphasize that resistance reduction effects similar as described here for funnel-
291 shaped channels must be expected in other partially widened pore structures as well.
292 Plasmodesmata with expanded central cavities, for example, appear quite common in leaf
293 tissues, and can often be found in thickened regions of the cell wall (Robinson-Beers and Evert
294 1991; Russin and Evert 1985; Oparka et al. 1999; Ehlers and Kollmann 2001). As the example in
295 Fig. 8 and previous studies (Blake 1978; Deinum et al. 2019) demonstrate, plasmodesmata of
296 such an architecture can be more conductive than cylindrical ones of the same minimum sleeve
297 width in much thinner portions of the wall. In funnel plasmodesmata, the very small opening
298 angles that suffice to halve hydraulic and diffusive resistance (Fig. 6A,B) represent structural

intricacies that probably will be missed on most TEM micrographs. Even if they were detected, they likely would be neglected in attempts to quantitatively model transport through these pores. Our results suggest that with regard to the efficiency of symplasmic transport, the idea that plasmodesmata can be adequately described as cylindrical or annular tubes might be a seriously misleading simplification. After all, a single funnel-shaped plasmodesma can be as conductive as dozens of cylindrical simple plasmodesmata taken together. In this light it appears necessary to collect precise ultrastructural data concerning plasmodesmata in various cell interfaces to understand and model symplasmic flow through plant tissues. Counting plasmodesma numbers and determining size exclusion limits appears insufficient.

Methods

Plant materials and growth conditions

Two monocots, *Triticum aestivum* and *Oryza sativa* (Poaceae), and the eudicots *Nicotiana tabacum* (Solanaceae), *Ipomoea nil* (Convolvulaceae), *Solenostemon scutellarioides* (Lamiaceae), *Catalpa speciosa* (Bignoniaceae), and *Medicago sativa* (Fabaceae) were grown from seeds in soil, and were maintained in a Greenhouse at 22°C, 60–70% relative humidity, and a 14 h light/10 h dark photoperiod (daylight augmented by 150 µmol m⁻² s⁻¹, Lamp Fixture #PL 90 cv PL Light Systems, Beamsville ON, Canada).

Sample fixation and embedding

Root tips harvested from mature plants were fixed and embedded based on Wu et al. (2012), but protocols had to be modified for some species. Excised root tips were fixed in 2% paraformaldehyde, 2% glutaraldehyde, 50 mM cacodylate buffer, pH 7 in a microwave oven (Biowave Pro, Pelco, Fresno CA, USA) at 750 W for two 90 s intervals on ice. The samples were

washed 3 times for 10 min with distilled water and then post-fixed overnight in 1% OsO₄. *I. nil*, *S. scutellarioides*, and *M. sativa* were again post-fixed in 2% OsO₄ for 2 hours, *C. speciosa* for 6 hours, and *T. aestivum* as well as *O. sativa* overnight to receive proper fixation in deeper cell layers. Samples were dehydrated in the microwave oven in a methanol series (up to 80% in 10% steps, 85%, 90%, 95%, 2 × 100% v/v, 1 min each) at 750 W irradiation. The methanol was replaced by propylene oxide in steps (50%, 2 × 100% v/v), and the samples were infiltrated with Spurr's resin (1:3, 1:2, 1:1, 2:1, 3 × pure resin for 1 day each). Embedded root tips were cured overnight at 60°C. Thin (70 nm) and semi-thin (220–280 nm) sections were produced with a Reichert Ultracut R ultramicrotome (Leica Microsystems, Wetzlar, Germany) and collected on formvar-coated Ni slot grids (Electron Microscopy Sciences, Hatfield PA, USA). Sections were stained with 2% uranyl acetate and 1% potassium permanganate for 12 min, followed by Reynold's Lead Citrate for 6 min. Semi-thin sections were post-stained with 1% tannic acid. 15 nm colloidal gold fudicals were precipitated on both grid surfaces by covering the grids with 5 μL solution for 10 min.

335 Transmission electron microscopy and plasmodesma geometry

336 Micrographs and tomograms were produced using a 200 kV Tecnai G2 20 Twin transmission
337 electron microscope (Thermo Fisher, Waltham MA, USA) equipped with an LaB6 filament and
338 an FEI Eagle 4k CCD camera. Structural parameters including plasmodesma length (L ;
339 equivalent to cell wall thickness) and the diameters of the plasmodesmal apertures on the sides of
340 the protophloem sieve element and the phloem pole pericycle cell (D_{PSE} and D_{PPP} , respectively)
341 were determined, as far as possible, on electron micrographs using ImageJ/Fiji IJ 1.46r
342 (<https://imagej.nih.gov/ij>). The opening angle (θ) of the cell wall pore was estimated as

$$\theta = \text{atan} \left(\frac{D_{\text{PSE}} - D_{\text{PPP}}}{2L} \right) \quad (1)$$

Because D_{PPP} could not be determined unambiguously on many micrographs, θ was calculated for $D_{\text{PPP}} = 20 \text{ nm}$ as well as $D_{\text{PPP}} = 50 \text{ nm}$, to cover the range of realistic values. Potential correlations between structural parameters were visualized as Geometric Mean Functional Relationships (GMFR; Draper and Smith 1998).

348 Electron tomography and 3D modeling

Using the transmission electron microscope described above, tilt series ($+55^\circ$ to -55° in 2° steps) were captured along two single-tilt orthogonal axes with the automated tomography acquisition suite Xplore3D (Thermo Fisher). Raw stacks were combined into dual-axis tomograms (Mastronarde 1997; Kremer et al. 1996) with the open source package IMOD 4.9 (<https://bio3d.colorado.edu/imod/>). Plasmodesmal tomograms were manually partitioned into two-material image segments comprising the desmotubule and inner pore volume with Amira 6.7 (Thermo Fisher). Tomography surface reconstructions were transferred into tetrahedral grid reconstructions with no more than 18000 triangles and used as input files for the flow simulation software COMSOL Multiphysics 5.4 (COMSOL Multiphysics, Burlington MA, USA).

358 Theory and computational analysis

We used a combination of numerical simulations and theory to evaluate the transport properties of funnel plasmodesmata. The analysis of diffusion was based on Fick's law, which links the flux, j , the concentration gradient ∇c , and the diffusion constant D (which for modeling purposes was assumed to be $6.7 \times 10^{-10} \text{ m}^2\text{s}^{-1}$):

$$j = -D \nabla c \quad (2)$$

364 Assuming solute conservation and steady-state conditions leads to the diffusion equation

365
$$\nabla^2 c = 0 \quad (3)$$

366 Transport characteristics of plasmodesmatal geometries extracted from Amira 6.7 were modeled
367 using COMSOL Multiphysics 5.4. The diffusion current I was determined by integrating the flux
368 across the pore entrance. The diffusion resistance

369
$$R_d = \frac{\Delta c}{I} \quad (4)$$

370 was computed where Δc is the imposed cell-to-cell concentration difference. In an idealized
371 concentric geometry, the current I can be expressed as

372
$$I = D\Delta c \left(\int_0^L A(x)^{-1} dx \right)^{-1} \quad (5)$$

373 where L is the length of the pore and $A(x)$ is the cross-sectional area of the open space in the
374 pore, measured as a function of the distance along the pore axis, x . For models with a central rod
375 of constant diameter b mimicking the desmotubule as shown in Fig. 5A, the outer radius a also is
376 a function of x :

377
$$a(x) = a_0 + x \tan \theta \quad (6)$$

378 where a_0 refers to the smaller, or outlet aperture and θ is the pore angle (see Fig. 5A).
379 Consequently, the cross-sectional area A of the space available for transport, or sleeve, changes
380 along the pore axis x according to

381
$$A(x) = \pi(a(x)^2 - b^2) \quad (7)$$

382 Combining Eqs. (4) and (5) leads to an expression for the diffusion resistance:

383
$$R_d = \frac{\Delta c}{I} = \frac{1}{D} \left(\int_0^L A(x)^{-1} dx \right) \quad (8)$$

384 The analysis of bulk flow through the pores was based on the Stokes equation,

385
$$\eta \nabla^2 \mathbf{v} = \nabla p, \quad (9)$$

386 where v is the velocity field, p is pressure, and η is the cytoplasmic viscosity (for modeling
387 purposes, η was set to 8.9×10^{-4} Pa s). A pressure drop Δp was applied across the pore and we
388 assumed no-slip conditions ($v = 0$) on all solid boundaries.

389 Flow characteristics of plasmodesmatal geometries extracted from Amira 6.7 were modeled
390 using COMSOL Multiphysics 5.4. Validation of the solver was carried out as described by
391 Jensen et al. (2012). The volumetric flow rate Q was determined by integrating the velocity field
392 across the pore entrance. Subsequently, the hydraulic resistance

393
$$R_h = \frac{\Delta p}{Q} \quad (10)$$

394 was computed. In an idealized concentric, conical geometry, the hydraulic resistance can be
395 expressed as

396
$$R_h = \frac{8\eta}{\pi} \int_0^L \left(a(x)^4 - b^4 - \frac{(a(x)^2 - b^2)^2}{\ln(a(x)/b)} \right)^{-1} dx \quad (11)$$

397 Both the numerical solutions of the COMSOL flow/diffusion analyses and physical models
398 comparing plasmodesmatal structural elements to theoretical flow profiles were examined using
399 MATLAB R2020b (MathWorks, Natick MA, USA).

400

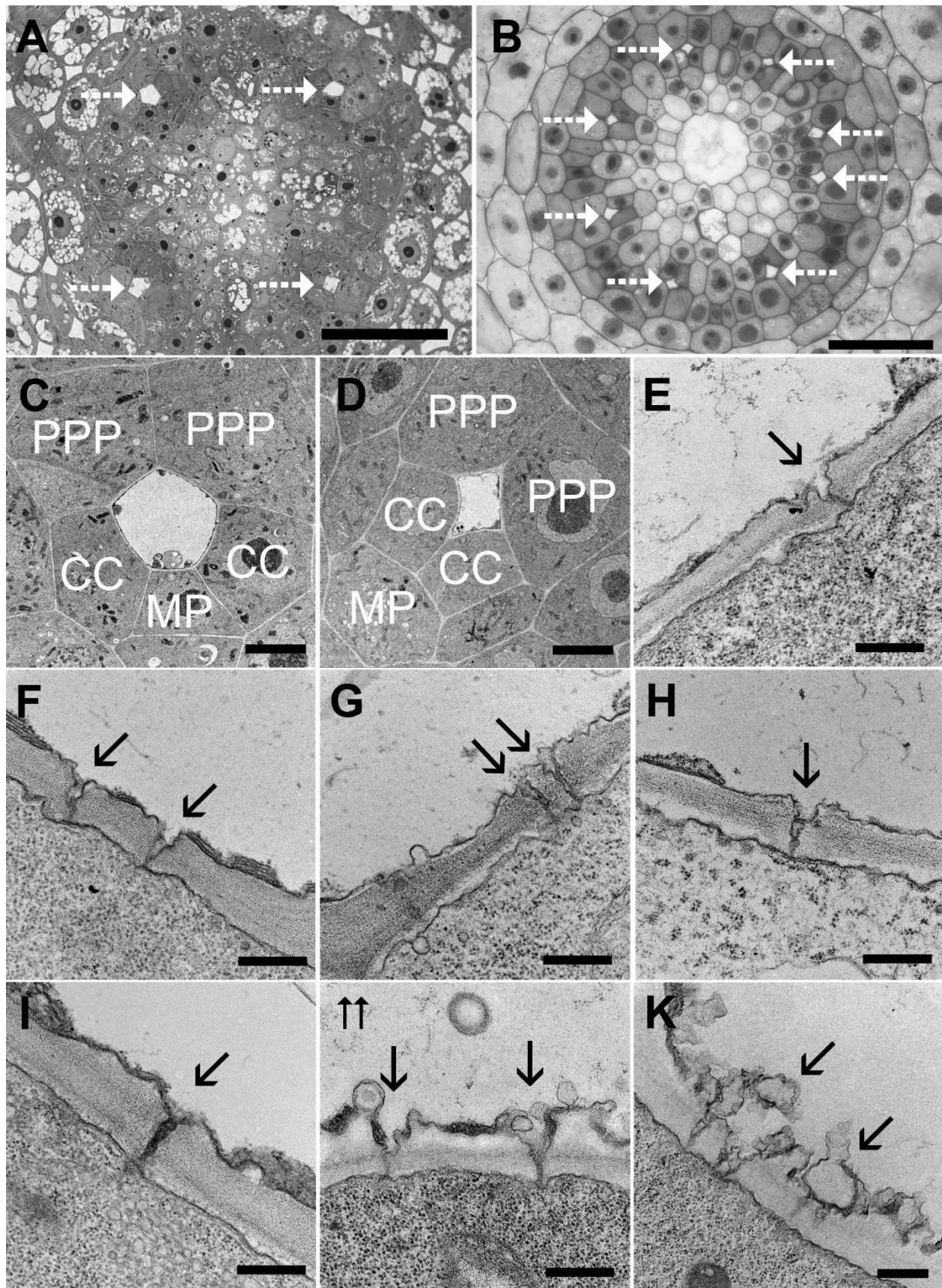
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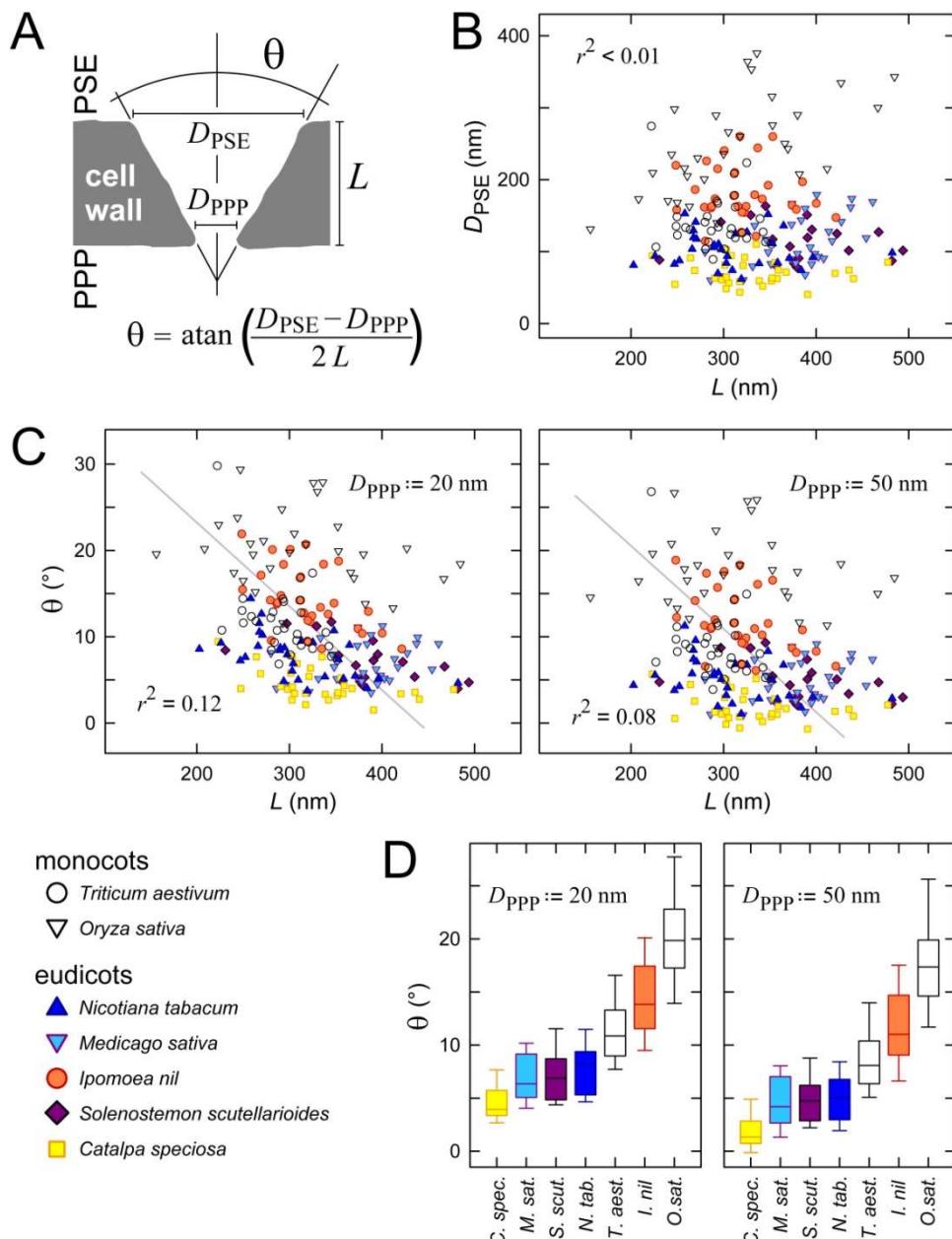
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503 **Figure 1: Tissue structure and funnel plasmodesmata in phloem unloading zones of growing root tips.**

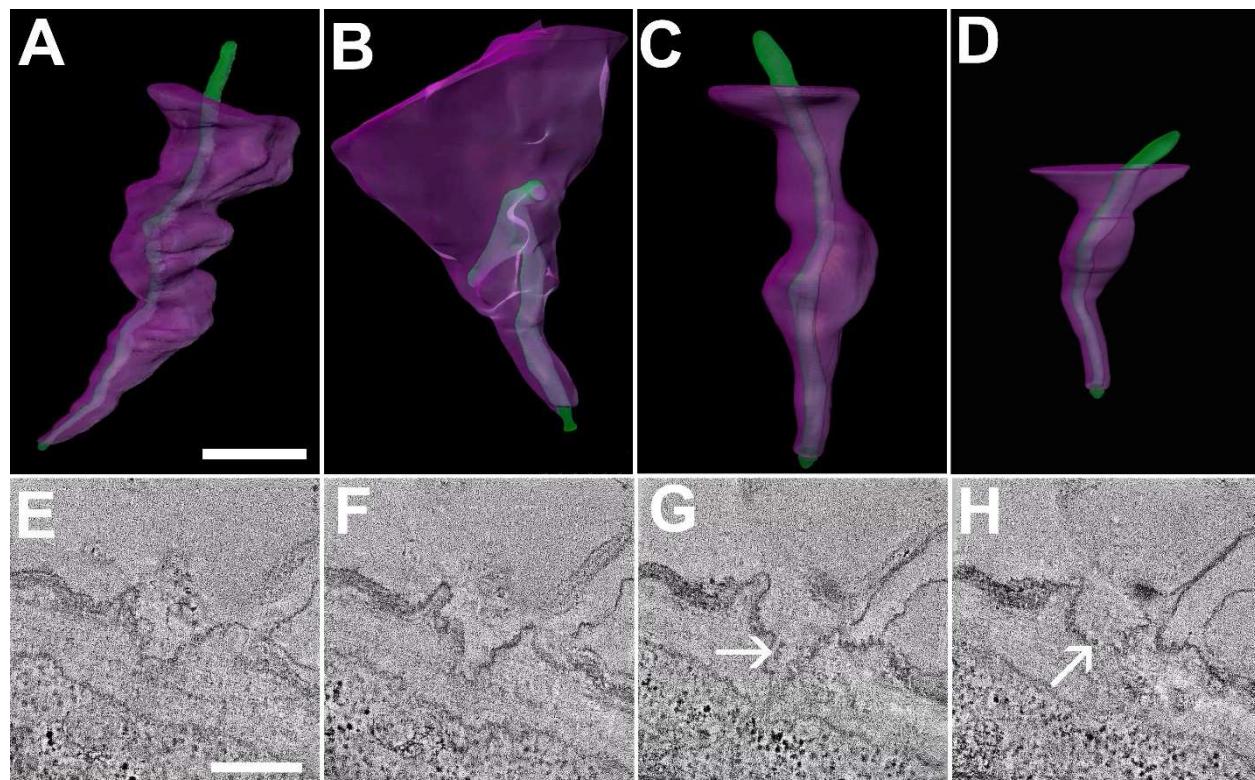
504 A) Cross-section of the central cylinder in the phloem unloading zone in a root of *Ipomoea nil*,
505 representative of the eudicots examined. The tetrarch vascular system has four protophloem sieve
506 elements, which appear empty due to the absence of dense cytoplasm (dashed arrows). B) Analogous
507 section of a polyarch root of *Triticum aestivum* here with eight protophloem sieve elements (dashed
508 arrows). C) Protophloem sieve elements typically connect to two companion cells (CC), two phloem pole
509 pericycle cells (PPP), and one immature metaphloem sieve element (MP) in eudicots, shown here in
510 *Ipomea nil*. D) In phloem unloading zones of the Poaceae roots tested, immature metaphloem sieve
511 elements do not reach the protophloem sieve elements, leaving them with only four direct neighbors.
512 The example shown is from *Oryza sativa*. In both (C) and (D), straight cell walls and the coherent
513 structure of the cytoplasm indicate excellent preservation. E-K) Funnel plasmodesmata (solid arrows) in
514 the PSE/PPP interfaces of five eudicots (E, *Ipomoea nil*; F, *Nicotiana tabacum*; G, *Catalpa speciosa*; H,
515 *Solenostemon scutellarioides*; I, *Medicago sativa*) and two Poaceae (J, *Oryza sativa*; K, *Triticum*
516 *aestivum*). Scale bars: A,B: 100 µm; C,D: 5 µm; E-H: 400 nm; K: 500 nm.



517

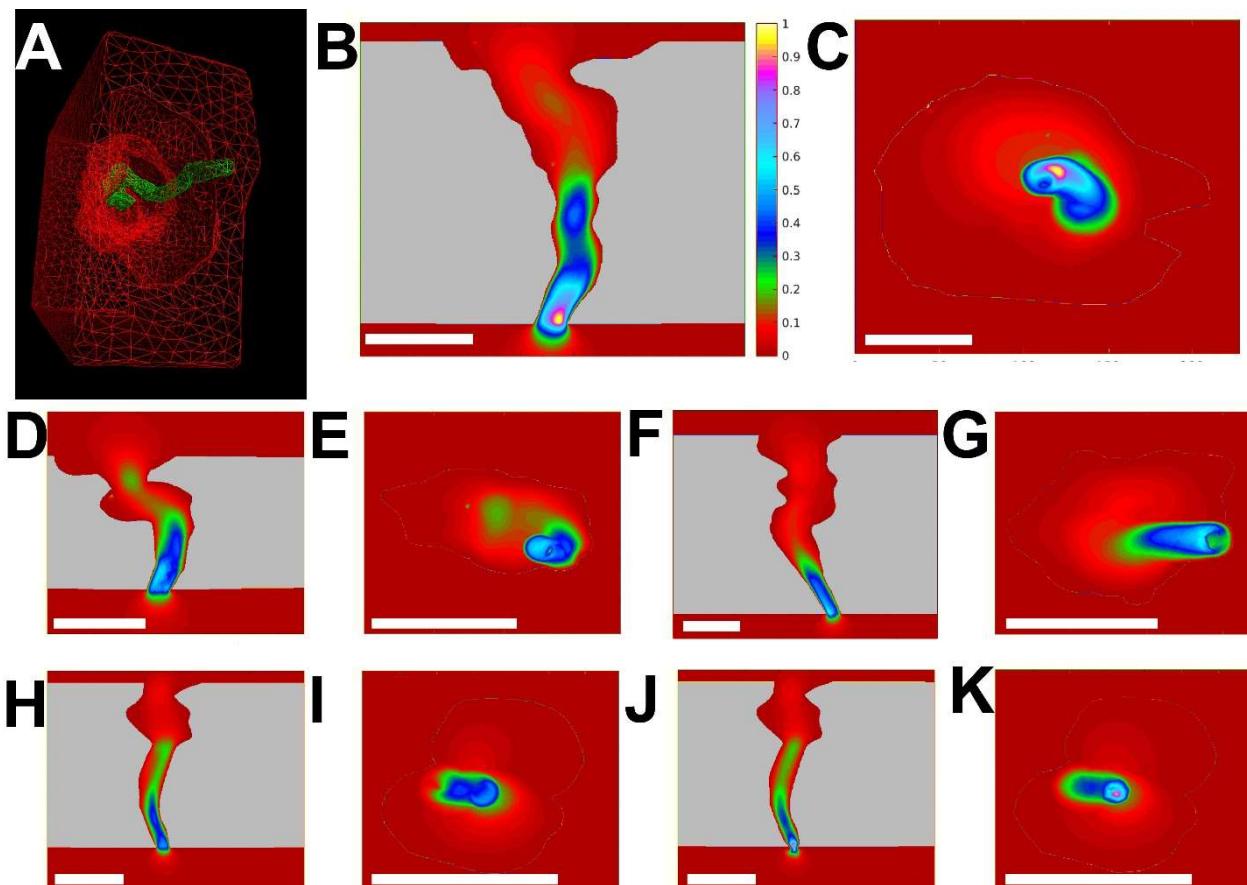
518 **Figure 2: Cell wall pore geometry in the PSE/PPP interfaces of different species.** (A) Pore opening
 519 angles (θ) were computed based on measurements taken on electron micrographs of plasmodesma
 520 length (equivalent to cell wall thickness), L , and the aperture diameter on the side of the protophloem
 521 sieve element, D_{PSE} . The diameter of the aperture facing the phloem pole pericycle, D_{PPP} , was set to 20 or
 522 50 nm. (B) No correlation existed between measurements of D_{PSE} and L ($r^2 < 0.01$). (C) Relation between

523 L and pore opening angle θ , assuming a D_{PPP} of 20 nm (left) and 50 nm (right). Grey lines indicate the
524 Geometric Mean Functional Relationship (GMFR). (D) Distribution of pore opening angles θ in different
525 species (D_{PPP} set to 20 nm and 50 nm as indicated). Boxes represent the second and third quartile of the
526 data with the median given as a horizontal line; whiskers indicate the 10th and 90th percentiles. The
527 number of plasmodesmata analyzed was $n = 18$ for *S. scutellarioides* and $n = 30$ for all other species (see
528 Supplemental Table S1 for original data).



529

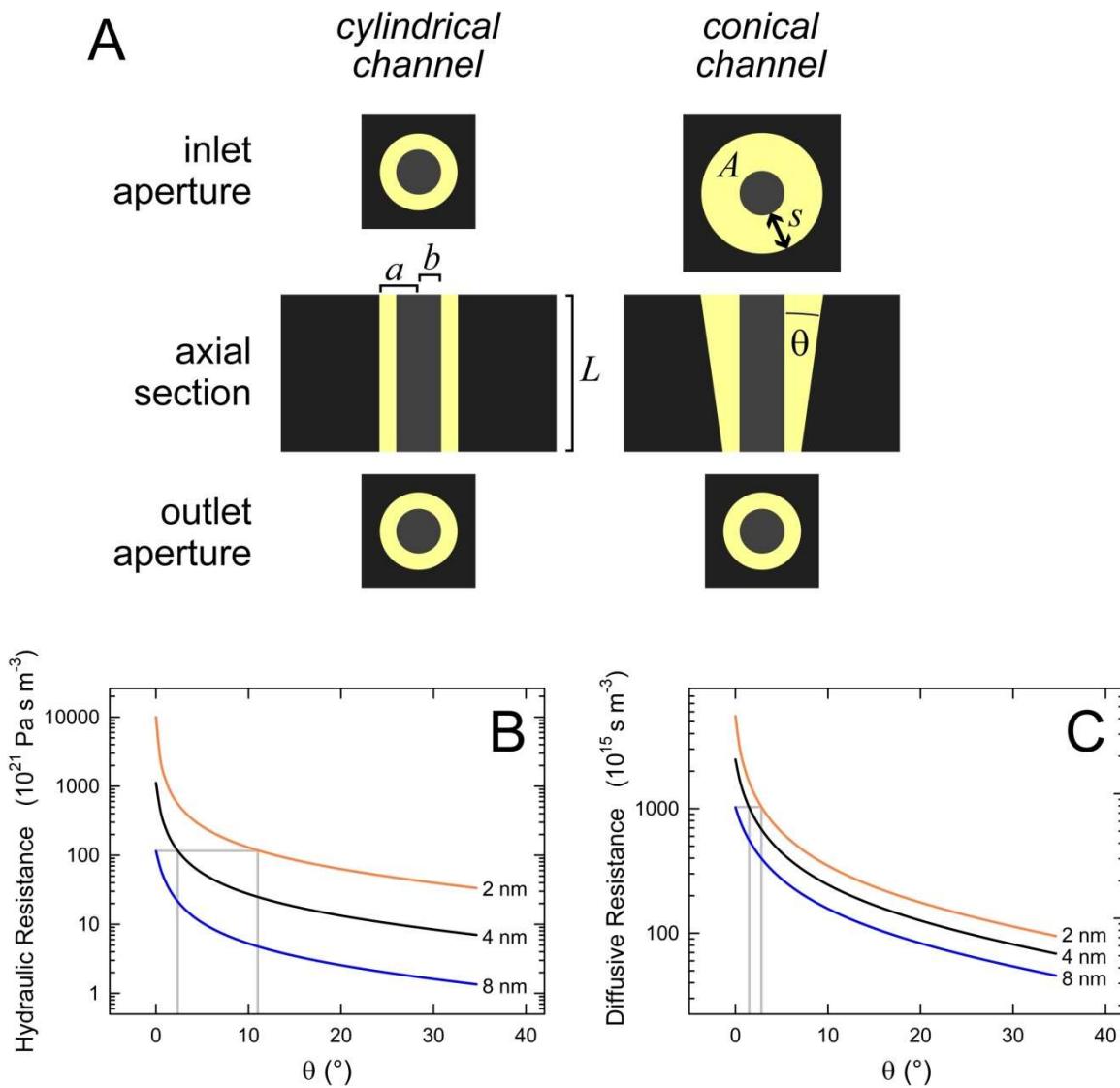
530 **Figure 3: 3D reconstructions of funnel plasmodesmata.** Electron tomography reconstructions of funnel
531 plasmodesmata from (A) *Triticum aestivum*, (B) *Oryza sativa*, (C) *Medicago sativa*, and (D) *Arabidopsis*
532 *thaliana*. The lumen of the cell wall pore is rendered in magenta, while the desmotubule appears in
533 green. The shapes of the cell wall pores and their surface structures varied widely in all investigated
534 species. E-H) Four sections of an electron tomograph of a funnel plasmodesma in *Oryza sativa*. The exact
535 dimensions of the desmotubule (arrows) are difficult to determine because the contrast depends on
536 desmotubule location within the sample volume and other parameters. Scale in A = 100 nm
537 (representative for A-D). Scale E = 200 nm (representative for E-H).



538

539 **Figure 4: Flow velocity distributions in funnel plasmodesmata determined by fluid dynamics**
540 **simulations.** A) Tetrahedral grit construction of a *Triticum aestivum* funnel plasmodesma (red) including
541 the desmotubule (green), as an example of the input used for the COMSOL software to compute flow
542 patterns in the cytosolic space between plasma membrane and desmotubule. Longitudinal and cross-
543 sectional projections of flow patterns in funnel plasmodesmata of *Ipomoea nil* (B,C), *Oryza sativa* (D,E),
544 and *Triticum aestivum* (F,G). The color scale shows relative values of flow velocity ranging from
545 minimum (red) to maximum (yellow; see scale in B) in each graph. As could be expected, the fastest flow
546 velocities occurred in the narrowest parts of the wall pores, implying that the total hydrodynamic
547 resistance of funnel plasmodesmata depends mostly on the dimensions of the wall pores close to their
548 apertures into the phloem pole pericycle cells. (H-K) Funnel plasmodesma in *Catalpa speciosa*. The
549 electron tomograms did not fully resolve dimensions of the space between plasma membrane and

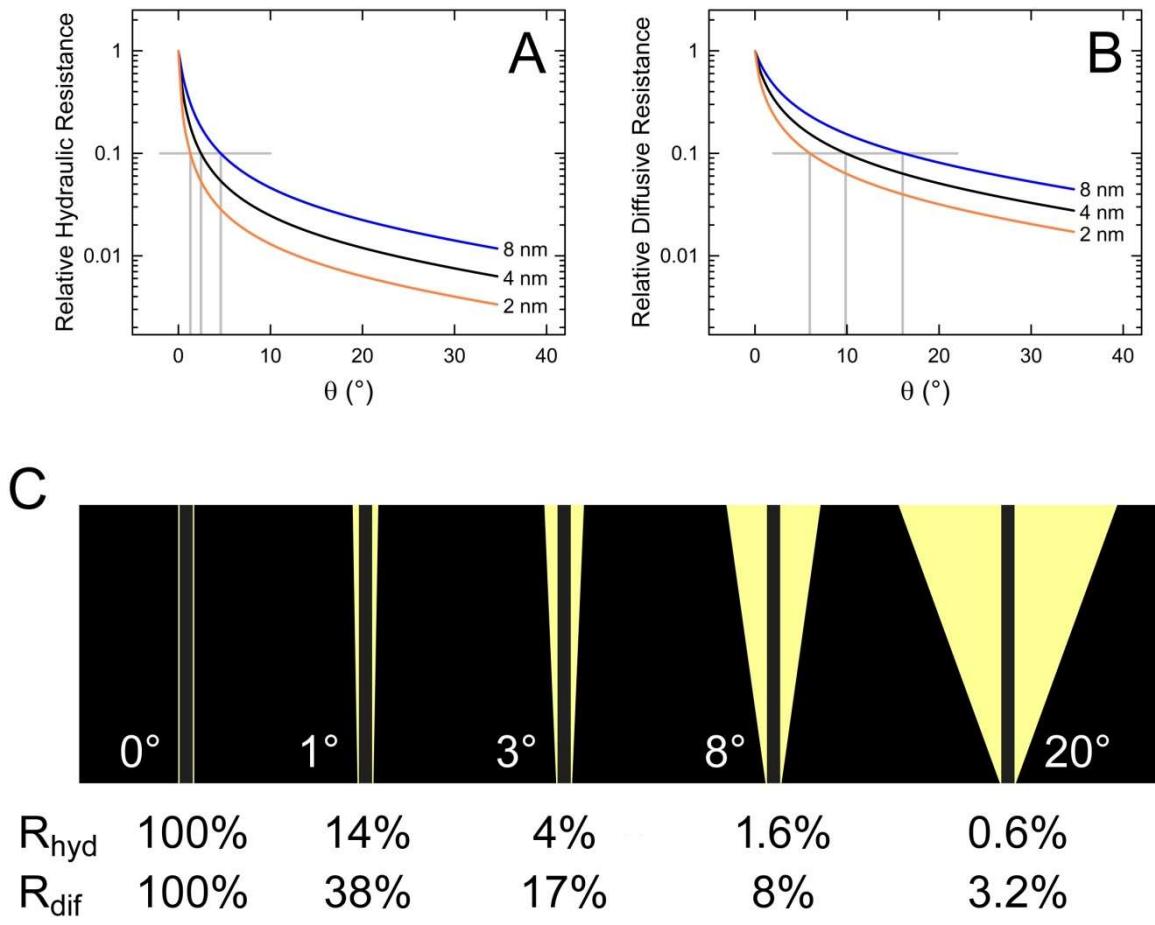
550 desmotubule. Manual adjustment of the diameter of this cytosol-filled sleeve to 4 nm (H,I) and 8 nm
551 (J,K) yielded almost identical patterns of flow velocity. Scale bar: 100 nm



552

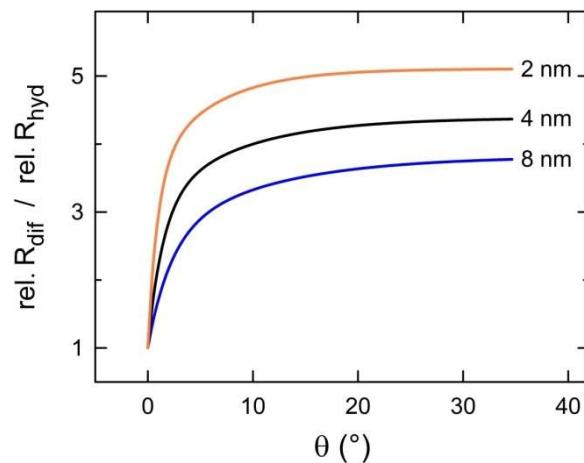
553 **Figure 5: Theoretical evaluation of the effects of channel geometry on hydraulic and diffusive**
 554 **resistance.** (A) Models of annular channels with radius a in walls of thickness L . Transport occurs only in
 555 the sleeve (yellow) between the outer channel wall and the central rod of radius b . The width of this
 556 sleeve (s) is constant in cylindrical channels (left) but increases steadily from the outlet to the inlet
 557 aperture in conical ones (right), as does the cross-sectional area (A) of the sleeve. Results shown below
 558 and in Fig. 6 were obtained with L (400 nm) and b (9.5 nm) kept constant, sleeve width at the outlet
 559 aperture (s_0) set to 2 (orange), 4 (black), or 8 nm (blue) by adjusting the pore radius at that position (a_0),

560 and angle θ between channel wall and central rod varying from 0° to 35° . (B) Absolute values of
561 hydraulic resistance as functions of θ . In cylindrical channels ($\theta = 0^\circ$), hydraulic resistance decreases
562 roughly ten-fold with every doubling of the sleeve width. However, channels with minimum sleeve
563 widths (s_0) of 2 and 4 nm reach the resistance of a cylindrical channel with an 8 nm sleeve at relatively
564 small θ , as indicated by grey lines. (C) Diffusive resistances as functions of θ ; details as in (B).



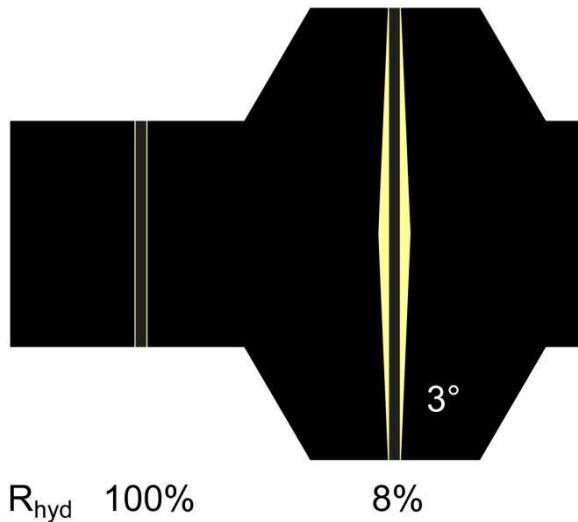
565

566 **Figure 6: Relative reductions of hydraulic and diffusive resistance in conical channels.** (A) Hydraulic
567 resistance of conical channels with varying θ and minimum sleeve widths of 2 (orange), 4 (black), and 8
568 nm (blue), normalized with respect to the resistance of a cylindrical channel of the corresponding sleeve
569 width. Grey lines highlight angles θ at which the resistance is reduced to 10% of that of conical channels.
570 (B) Dependence of diffusive resistance on θ ; details as in (A). (C) Examples of model channels drawn to
571 scale to visualize actual geometries; the minimum sleeve width at the outlet aperture (bottom) is 2 nm,
572 channel length is 400 nm. Values of hydraulic resistance (R_{hyd}) and diffusive resistance (R_{dif}) are given as
573 percentages of the resistances of the cylindrical control channel ($\theta = 0^\circ$; left).



574

575 **Figure 7: Ratios between the relative change in diffusive resistance (rel. R_{dif} ; from Fig. 6B) and the**
576 **relative change in hydraulic resistance (rel. R_{hyd} ; from Fig. 6A) at different pore angles.** With increasing
577 angle θ , the relative hydraulic resistance decreases more strongly than the relative diffusive resistance,
578 implying that convective processes become more important compared to diffusive processes as the
579 angle widens. The effect is stronger in narrower pores.



580

581 **Figure 8: Reduction of hydraulic resistance in a biconical channel.** Plasmodesmata with central
582 widenings are often found in thickened portions of the cell wall. This example compares the hydraulic
583 resistance of a biconical model channel (right) with opening angle $\theta = 3^\circ$ and apertural sleeve width 2
584 nm in a thickened cell wall (800 nm) to that of a cylindrical channel (left) with 2 nm sleeve width in a
585 wall of 400 nm thickness. Model channels are drawn to scale to visualize actual geometries.