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1 **The causes of leaf hydraulic vulnerability and its influence on** 2 **gas exchange in *Arabidopsis thaliana***

3 Christine Scoffoni^{1,2}, Caetano Albuquerque³, Hervé Cochard⁴, Thomas N. Buckley⁵, Leila R.
4 Fletcher¹, Marissa A. Caringella¹, Megan Bartlett⁶, Craig R. Brodersen⁷, Steven Jansen⁸, Andrew
5 J. McElrone^{3,9}, Lawren Sack¹

6 ¹ *Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621*
7 *Charles E. Young Drive South, Los Angeles, California 90095, USA*

8 ² *Department of Biological Sciences, California State University, Los Angeles, 5151 State*
9 *University Drive, Los Angeles, California 90032, USA*

10 ³ *Department of Viticulture and Enology, University of California, Davis, CA 95616, USA*

11 ⁴ *Université Clermont-Auvergne, INRA, PIAF, F-63000 Clermont-Ferrand, France*

12 ⁵ *Department of Plant Sciences, University of California, Davis, CA 95616, USA*

13 ⁶ *Princeton Environmental Institute, Princeton University, NJ 08544, USA*

14 ⁷ *School of Forestry & Environmental Studies, Yale University, 195 Prospect Street, New Haven,*
15 *CT 06511, USA*

16 ⁸ *Institute of Systematic Botany and Ecology, Ulm University*

17 ⁹ *USDA-Agricultural Research Service, Davis, CA 95616, USA*

18
19 **One-sentence summary:** Declines in leaf outside-xylem hydraulic conductance prior to turgor
20 loss point contribute strongly to stomatal closure, and improve performance, survival and
21 efficient water use during drought.

22
23 **Author contributions:** C. Scoffoni and L. Sack designed the study. C. Scoffoni, C.
24 Albuquerque, H. Cochard, T.N. Buckley, L.R. Fletcher, M. Caringella, M.K. Bartlett, A.J.
25 McElrone & L. Sack contributed to data collection and/or analyses. C. Scoffoni and L.
26 Sack wrote the manuscript with input from all authors.

27 28 **Corresponding Author:**

29 Christine Scoffoni

30 California State University, Los Angeles

31 Department of Biological Sciences

32 5151 State University Drive, Los Angeles 90032, California

33 Email: cscoffo@calstatela.edu

34

35

36 **Abstract**

37 The influence of the dynamics of leaf hydraulic conductance (K_{leaf}) diurnally and during
38 dehydration on stomatal conductance and photosynthesis remains unclear. Using the model
39 species *Arabidopsis thaliana* (ecotype Col-0), we applied a multi-tiered approach including
40 physiological measurements, high-resolution X-ray micro-computed tomography, and modelling
41 at a range of scales to characterize: (1) K_{leaf} decline during dehydration; (2) its basis in the hydraulic
42 conductances of leaf xylem (K_x) and outside-xylem pathways (K_{ox}); (3) the dependence of its
43 dynamics on irradiance; (4) its impact on diurnal patterns of stomatal conductance and
44 photosynthetic rate; and (5) its influence on gas exchange and survival under simulated drought
45 regimes. *Arabidopsis* leaves showed strong vulnerability to dehydration diurnally in both gas
46 exchange and hydraulic conductance, despite lack of xylem embolism or conduit collapse above
47 turgor loss point, indicating pronounced sensitivity of K_{ox} to dehydration. K_{leaf} increased under
48 higher irradiance in well-hydrated leaves across the full range of water potential, but no shift in
49 K_{leaf} vulnerability was observed. Modelling indicated that responses to dehydration and irradiance
50 are likely attributable to changes in membrane permeability, and that a dynamic K_{ox} would
51 contribute strongly to stomatal closure, improving performance, survival and efficient water use
52 during drought. These findings for Col-0 provide a baseline for assessing variation across
53 genotypes in hydraulic traits and their influence on gas exchange during dehydration.

54

55 **Key words:** aquaporins, drought, leaf water relations, plant modelling, pv-curves

56 Introduction

57 Plant growth requires a copious water supply because the rate of CO₂ uptake for photosynthesis
58 depends on stomatal conductance, which results in transpiratory water loss. Because stomata close
59 in dehydrating leaves, photosynthesis and growth depend on the efficiency of water replacement
60 to the mesophyll. Thus, in the past two decades, many studies focusing on diverse species have
61 shown the centrality of the plant hydraulic system in determining leaf-scale gas exchange and plant
62 productivity (Sack and Holbrook, 2006; Brodribb et al., 2007; Scoffoni et al., 2016). Our aim was
63 to test hypotheses for the dynamics of hydraulic traits and their influence on gas exchange during
64 dehydration using the model species *Arabidopsis*. Establishing a framework for testing the
65 influence of hydraulic traits in *Arabidopsis* can help address recent debates and open avenues for
66 discovery of genetic associations in natural and mutant genotypes under moist conditions and
67 during soil and/or atmospheric drought.

68 The leaf accounts for a large proportion of plant hydraulic resistance (Sack and Holbrook,
69 2006). Thus, theoretical and empirical studies have shown strong correlations of stomatal
70 conductance (g_s) and photosynthetic rate (A_{max}) with leaf hydraulic conductance (K_{leaf} ; determined
71 as the flow rate divided by water potential driving force, in units $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) across species
72 under well-watered conditions (Nardini and Salleo, 2003; Brodribb and Holbrook, 2004; Sack and
73 Holbrook, 2006; Scoffoni et al., 2016), and within given species during dehydration (Brodribb and
74 Holbrook, 2006, 2007; Bartlett et al., 2016; Scoffoni and Sack, 2017). A high K_{leaf} enabling higher
75 g_s and A_{max} could be achieved through a high vein length per area, larger and/or more numerous
76 xylem conduits (and/or xylem pits), and more conductive mesophyll and bundle sheath anatomy
77 and biochemistry (Brodribb et al., 2007; Choat et al., 2008; Caringella et al., 2015; Scoffoni et al.,
78 2015; Scoffoni et al., 2016; Stewart et al., 2018). Yet, the linkages of K_{leaf} and gas exchange as
79 leaves dehydrate to turgor loss point are still under debate. Early studies suggested that K_{leaf} decline
80 drives stomatal closure under high vapor pressure deficits at mid-day (Brodribb and Holbrook,
81 2003a; Bucci et al., 2003) and during drought (Salleo et al., 2001; Brodribb and Holbrook, 2003b;
82 Nardini and Salleo, 2003). Several recent studies suggested that in some species, K_{leaf} might not
83 decline until embolism forms in the leaf vein xylem (Brodribb et al., 2016a; Brodribb et al., 2016b;
84 Skelton et al., 2017), which for many species does not occur until past the point of stomatal closure
85 and bulk leaf turgor loss (Brodribb et al., 2016b; Hochberg et al., 2017; Scoffoni et al., 2017a).
86 Similarly, xylem wall collapse may drive K_{leaf} declines in pine needles and minor veins of an oak

87 species, but only below turgor loss point (Cochard et al., 2004a; Zhang et al., 2016). Avoiding K_{leaf}
88 decline during transpiration when leaves are hydrated above turgor loss point has been suggested
89 as adaptive, maintaining leaf water potential and open stomata, though at the risk of sustaining
90 water potentials that would induce xylem cavitation under high vapor pressure deficits (Brodribb
91 and Holbrook, 2006). Numerous studies in the last decade have shown that species differ in
92 whether K_{leaf} declines at milder, similar or more severe leaf water potentials than at stomatal
93 closure, and that K_{leaf} decline depends mechanistically on processes in multiple tissues—the
94 venation, bundle sheath, and mesophyll pathways of liquid and vapor transport (reviewed by
95 Scoffoni and Sack, 2017). Indeed, a meta-analysis of the literature found that on average, across
96 species (and methods for K_{leaf} determination), K_{leaf} declined by 30-80% before turgor loss point
97 (Scoffoni and Sack, 2017). Recent work focusing on partitioning leaf xylem and outside-xylem
98 resistances during dehydration suggested the outside-xylem hydraulic conductance (K_{ox}) as the
99 primary driver of K_{leaf} decline (Trifilo et al., 2016; Scoffoni et al., 2017a; Scoffoni and Sack, 2017),
100 which could be triggered by the loss of cell connectivity, cell shrinkage, and/or changes in
101 membrane aquaporin activity (Laur and Hacke, 2014b; Scoffoni et al., 2014; Scoffoni et al.,
102 2017a), potentially mediated by effects of ABA in the bundle sheath (Pantin et al., 2013). A recent
103 study in rice has attributed to K_{leaf} decline a strong causal role in driving stomatal closure during
104 dehydration (Wang et al., 2018).

105 Debate has also focused on the light response of K_{leaf} . Previous studies have found many
106 species to exhibit a rapid enhancement of K_{leaf} in response to increased irradiance (Sack et al.,
107 2002; Nardini et al., 2005b; Tyree et al., 2005; Cochard et al., 2007; Scoffoni et al., 2008; Voicu
108 et al., 2008; Guyot et al., 2012; Xiong et al., 2018), but not all (Sack et al., 2002; Gasco et al.,
109 2004; Tyree et al., 2005; Scoffoni et al., 2008; Xiong et al., 2018). Activation of PIP_{2,1} and PIP_{2,2}
110 aquaporins under high irradiance at high water potential has been shown to also enhance K_{leaf} in
111 some (Cochard et al., 2007), though not all species (Voicu et al., 2009). A higher K_{leaf} under high
112 light could potentially help buffer rapid changes in VPD and prevent stomata from closing (Cairns
113 Murphy et al., 2012; Scoffoni et al., 2015). In *Arabidopsis*, one study estimated hydraulic
114 conductance by pushing water into entire rosettes suspended underwater in a dark pressure
115 chamber, and found it was higher for leaves acclimated to dark rather than high irradiance (Prado
116 et al., 2013), though no study has investigated this response at the leaf level.

117 Here, we applied complementary physiological, imaging, and modelling approaches (Table
 118 1) to assess K_{leaf} dynamics with dehydration and irradiance, and their role in driving diurnal
 119 patterns of gas exchange in Arabidopsis. We tested the hypotheses in Arabidopsis that K_{leaf} : (1) is
 120 high under well-hydrated conditions, but declines strongly during dehydration; (2) declines due to
 121 changes in K_{ox} but not xylem embolism formation or conduit collapse; (3) responds to irradiance;
 122 (4) influences diurnal patterns of stomatal conductance and photosynthetic rate; and (5) shows
 123 dynamics that confer higher water-use efficiency, and that would thus benefit plant performance
 124 under simulated soil drying.

125

126 Results

127 *Leaf hydraulics and gas exchange and their responses to leaf dehydration and irradiance in* 128 *Arabidopsis*

129 Arabidopsis Col-0 exhibited high maximum leaf hydraulic conductance (K_{leaf}), stomatal
 130 conductance (g_s), minimum epidermal conductance (g_{min}), as well as light-saturated photosynthetic
 131 rate (A_{area}) (Figure 1, Figure 2, Table 2). The partitioning of hydraulic resistances in the leaf
 132 indicated a similar distribution of resistances in the xylem and outside-xylem pathways (45.6 vs.
 133 54.4% respectively; Table 2).

134 Arabidopsis showed a strong vulnerability to dehydration in K_{leaf} and gas exchange (Figure
 135 1). Notably, the range of water potential measured on intact plants diurnally, and on detached
 136 leaves during bench dehydration was similar (Figure 1, Figure 2). K_{leaf} responded non-linearly to
 137 dehydration, with steep declines before 50% loss of its initial K_{leaf} by -0.17 MPa ($K_{\text{leaf}} P_{50}$), and
 138 gradually slowing down its response to further dehydration (Table 2, Figure 1). Both g_s and A_{area}
 139 responded linearly to declining Ψ_{leaf} (Figure 2), reaching 50% loss of initial rates by -0.37 and $-$
 140 0.38 MPa respectively, and 95% loss at similar Ψ_{leaf} values of -0.71 MPa (Table 2). At turgor loss
 141 point, K_{leaf} had declined by ca. 88%, and stomata were nearly fully closed (Table 2, Figure 1).

142 Leaves acclimated to high irradiance had significantly higher K_{leaf} values than leaves
 143 acclimated to low irradiance, with a 60% enhancement of K_{leaf} from low to high irradiance in well-
 144 hydrated leaves of Col-0. (Figure 1; *t*-test done on residuals of K_{leaf} , i.e., difference of observed
 145 values relative to those predicted from the best fit function through all data combined: ($K_{\text{leaf}} =$
 146 $8.33 + 83.7 \times \exp(-(9.47 \times \Psi_{\text{leaf}}))$). Residuals for K_{leaf} were $7.4 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ higher
 147 under high irradiance across the entire vulnerability curve ($p = 0.01$), $7.9 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ higher

148 considering only leaves above turgor loss point ($p = 0.01$), and $13.9 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ higher
149 considering only leaves at hydration above -0.2 MPa ($p = 0.04$). However, leaves acclimated to
150 high and low irradiance were similar in their $K_{\text{leaf}} P_{50}$ (-0.17 vs. -0.16 MPa respectively; Figure 1).

151

152 *Diurnal responses of gas exchange*

153 Photosynthetic and stomatal responses were measured over the course of two days, from 0900 to
154 1800. Our results showed that the diurnal pattern of whole-plant hydraulic conductance and gas
155 exchange reflected the dynamics of Ψ_{leaf} , as evidenced by the strong trends of K_{plant} , g_s and A_{max}
156 versus Ψ_{leaf} ($r^2 = 0.45-0.81$; $p < 0.02$; Figure 2). Of all the potential environmental drivers, vapor
157 pressure deficit (VPD) most strongly correlated with g_s dynamics diurnally ($r^2 = 0.18$; $p = 0.002$;
158 Supplemental Figure S1).

159 Independent effects analysis of potential drivers of diurnal dynamics in g_s , including
160 environmental factors and Ψ_{leaf} showed that Ψ_{leaf} was the most important statistically, contributing
161 77% towards the diurnal variation (Supplemental Figure S2). The VPD contributed 11%, and
162 temperature, PAR and time of day each contributed only 4% to the observed variation
163 (Supplemental Figure S2).

164

165 *Testing for vein xylem embolism and collapse during leaf dehydration using micro-computed* 166 *tomography*

167 We scanned leaves using *in vivo* micro-computed tomography for dehydrated plants to visualize
168 potential xylem embolism. In 14/18 leaves attached to plants that spanned the observed range of
169 Ψ_{leaf} (-0.05 to -0.87 MPa), no gas embolism was observed in major or minor veins (Figure 3). In
170 4/18 scans, we observed 1-2 embolized conduits in the midrib and/or secondary veins; notably,
171 these leaves were not the most dehydrated ($\Psi_{\text{leaf}} = -0.13$ to -0.45 MPa ; Figure 4; Table 3) but were
172 within the same range as other leaves that did not exhibit embolism. In all three leaves that showed
173 embolized midrib conduits, the embolism spanned the entire length of the scanned section, and we
174 were unable to measure the total vessel length (Figure 4; Table 3). For two leaves, the embolized
175 midrib conduit extended into a secondary vein. In the fourth leaf, an isolated embolized conduit in
176 the secondary vein was observed (Figure 4, Table 3). All embolized conduits were of average
177 diameter (Table 3; midrib conduit diameters measured under light microscopy ranged from 2.79
178 to $10.3 \mu\text{m}$). No collapsed conduits were observed in midrib and secondary vein conduits at the

179 range of water potentials investigated. The resolution of the micro-CT scans was not sufficient to
180 determine whether conduit collapse occurred in higher-order veins.

181

182 *Modelling the impact of embolism and collapse on K_x*

183 Spatially explicit modelling of the leaf xylem (Table 1) showed that the very low level of observed
184 xylem conduit embolism would reduce leaf xylem hydraulic conductance (K_x) by 1.2 to 4.7%
185 (Table 3). Because resolution was not sufficient to determine whether conduit collapse occurred in
186 higher-order veins, we simulated the potential impact of such collapse if it had occurred. These
187 simulations showed that if higher-order veins were to collapse to the same % of conduit diameter
188 as recently reported for minor veins of *Quercus rubra* (Zhang et al., 2016), this would decrease K_x
189 by 3-7.5% (Table 3). Under a more extreme scenario in which collapse of tertiary and minor veins
190 caused a 50% decline in their conductivity, K_x would be reduced by 12-17% (Table 3), which
191 would decrease K_{leaf} by 7- 9%.

192

193 *Modelling the putative causes of K_{ox} decline*

194 Spatially explicit modelling of the outside-xylem pathways using MOFLO 2.0 (Table 1) suggested
195 that the main factor accounting for the decline in K_{ox} observed at -0.5 MPa was most likely
196 reduction of cell membrane permeability in combination with an apoplastic barrier at the bundle
197 sheath (Figure 5). Under high irradiance, an 80% reduction of cell membrane permeability would
198 cause a 68.4% decrease in K_{ox} ; adding an 80% reduction in cell connectivity would further decrease
199 K_{ox} by 0.2 % (Figure 5). When performing these simulations with no apoplastic barrier at the
200 bundle sheath, the impact of an 80% reduction of cell membrane permeability caused only a 24.5%
201 decrease in K_{ox} (Figure 5). Simulating the impact of changes of temperature gradients due to light
202 absorption changed the percent loss of K_{ox} by 1-3% across simulations (Supplemental Table S1).
203 Finally, simulating the impact of cell shrinkage from full turgor to -0.5 MPa resulted in an increase
204 in K_{ox} by 7 to 15%, due to the increase in vein density caused by leaf shrinkage and the consequent
205 decrease in outside-xylem water flow pathlengths (Figure 5).

206

207 *Partitioning the contribution of K_{leaf} vulnerability to g_s decline*

208 In a transpiring leaf, a low Ψ_{leaf} would result from low water potentials proximally to the leaf (i.e.,
209 in the soil or roots; Table 1), and to the transpiration-driven water potential drop across the leaf,

210 which is greater, given K_{leaf} vulnerability. Thus, given that g_s declines with Ψ_{leaf} , K_{leaf} vulnerability
211 will amplify the reduction of g_s at a given soil water potential and vapor pressure deficit. Using a
212 partitioning analysis, we applied the observed parameters of g_s and K_{leaf} decline in *Arabidopsis* to
213 compute the marginal % contribution of K_{leaf} vulnerability to the decline of g_s (Table 1). Our results
214 showed that K_{leaf} vulnerability contributes strongly to g_s decline in transpiring leaves early in
215 dehydration, due to amplification of Ψ_{leaf} decline; when g_s declines by 30%, 70% of this response
216 is due to K_{leaf} vulnerability rather than low water potential proximal to the leaf (Figure 6). The
217 contribution of K_{leaf} vulnerability to g_s decline remains >40% until g_s declined by 50%, and
218 becomes less important as stomata approach full closure. When g_s has declined by 95%, the
219 contribution of K_{leaf} vulnerability to g_s decline is < 1%.

220

221 *Using the SurEau whole-plant physiology model to estimate the influence of K_{leaf} decline on gas*
222 *exchange, productivity and survival*

223 We tested the importance of K_{leaf} vulnerability prior to turgor loss point in reducing g_s and
224 photosynthesis on plant carbon balance and survival in simulations using SurEau (Martin-StPaul
225 et al., 2017) (Figure 7A-D; Table 1). In simulations, the experimentally observed K_{leaf} vulnerability
226 caused an up to -0.36 MPa lower Ψ_{leaf} at midday under well-hydrated conditions (yellow and red
227 lines), compared to constant K_{leaf} simulations (light and dark blue lines) (Figure 7B). This lower
228 Ψ_{leaf} in turn reduced g_s and cumulative CO_2 assimilation ($A_{n, \text{tot}}$) by up to 62 and 17% respectively
229 under well-hydrated conditions (Figure 7A-B), but cumulative water-use efficiency (calculated as
230 $A_{n, \text{tot}}$ /total transpiration) increased by 28% (inset in Figure 7B). Given finite soil water supply in
231 these simulations, this higher water-use efficiency led to up to 24% higher $A_{n, \text{tot}}$ over the entire
232 course of the simulated drought. Indeed, because K_{leaf} vulnerability results in lower g_s during the
233 early stage of the drought, the soil water potential (approximated as nighttime Ψ_{leaf} in Figure 7B)
234 is maintained at higher levels as drought ensues, leading to the maintenance of higher g_s during
235 later drought (Figure 7A). Additionally, because Ψ_{leaf} does not drop as fast during the course of
236 the drought, these simulations showed that given K_{leaf} vulnerability, the onset of leaf xylem
237 embolism occurs later during drought such that plants survive up to 6 days longer under drying
238 soil (Figure 7D). These simulations resulted in similar findings whether or not K_{root} was set as
239 vulnerable or constant, highlighting K_{leaf} vulnerability as a main driver of improved water-use
240 efficiency, $A_{n, \text{tot}}$ and survival during soil drying.

241

242 *Drought tolerance in Arabidopsis*

243 *Arabidopsis* Col-0 exhibits low leaf mass per area, a high degree of area shrinkage during
244 dehydration, high minimum epidermal conductance (g_{min}), high osmotic potential at full turgor and
245 turgor loss point, low modulus of elasticity and relative water content at turgor loss point (Table
246 1).

247

248 **Discussion**

249 Our results demonstrate a potential strong role for outside-xylem pathways in the decline of K_{leaf}
250 with leaf dehydration, contributing to stomatal closure and the reduction of photosynthetic rate in
251 *Arabidopsis thaliana* (Col-0). Strong declines in K_{leaf} were associated with declines in K_{plant} , g_s and
252 A_{area} , at water potentials where no significant embolism was observed using microCT. The absence
253 of leaf xylem embolism before stomatal closure and hydraulic decline point to changes in outside-
254 xylem pathways as the cause of observed K_{leaf} decline and imply no functional role of xylem
255 dysfunction in this species' response of gas exchange to leaf dehydration. Modelling showed that
256 K_{leaf} vulnerability has a strong causal role in determining stomatal closure, and further, that K_{leaf}
257 vulnerability would improve plant carbon balance and survival during drought.

258

259 *Drivers of leaf hydraulic conductance decline during dehydration*

260 Our results suggest that changes in outside-xylem pathways are the main drivers of the response
261 of K_{leaf} to dehydration in *Arabidopsis*. MicroCT imaging showed that embolism was rare in major
262 vein xylem conduits and nonexistent in minor veins. Only one or two embolized conduits
263 (representing on average 6-11% of the conduits in the midrib) were found in 4/18 samples, with
264 no trend of embolism with increasing water stress prior to turgor loss point. This low vulnerability
265 to embolism in leaves parallels findings for *Arabidopsis* inflorescence stems, which have P_{50}
266 values lower than -2.5 MPa (Tixier et al., 2013). The few rare observed leaf vein xylem emboli
267 likely arose from methodological artifacts. In the 3/4 samples in which embolized conduits were
268 observed, the embolized conduit spanned the entire section. One possibility is that air may have
269 entered the conduit when plants were removed from the soil for dehydration, if air entered conduits
270 from damaged roots, and conduits were continuous into the major veins of scanned leaves.
271 Similarly, air may have entered when the two leaves were excised from the plant for initial water

272 potential measurement, if conduits spanned from these leaves to others in the rosette including the
273 scanned leaves. Alternatively, these few embolisms could be the result of defects in the
274 development of these conduits (Pickard, 1981; Tyree et al., 1994). Indeed, we found a single
275 isolated embolism event occurred in a secondary vein of one of our samples. Such isolated
276 embolism events have been reported in leaf veins of other angiosperm species (Scoffoni et al.,
277 2017b) and stem xylem (Brodersen et al., 2013; Choat et al., 2015; Choat et al., 2016).

278 MicroCT imaging did not reveal any conduit collapse in the midrib or secondary veins
279 across the range of observed water potentials. Since the resolution of the microCT imaging did not
280 permit assessment of xylem conduit collapse in higher-order veins, we tested the potential effect
281 of collapse of minor veins on K_{leaf} using a spatially explicit model of the leaf vein system. These
282 simulations suggested that if xylem conduit collapse in the tertiary and minor veins were to occur
283 within the range of water potentials in which K_{leaf} declined, this collapse would have a
284 quantitatively small effect, i.e., causing <10% decline in K_{leaf} at -0.5MPa. This finding was
285 consistent with previous model results showing that extreme collapse of minor veins would cause
286 K_{leaf} to decline only by up to 4% for four diverse species (Scoffoni et al., 2017b). Previous studies
287 found collapse of xylem conduits in pine needles and the minor veins of oak leaves, but only past
288 turgor loss point, and suggested this could act as a circuit breaker to protect the stem xylem from
289 embolism formation (Cochard et al., 2004a; Zhang et al., 2016). An early decline in outside-xylem
290 pathways would act in a similar way, hastening stomatal closure, before xylem collapse would
291 occur (Scoffoni et al., 2017a; and see following sections). Past turgor loss point, the Arabidopsis
292 leaf undergoes drastic shrinkage in area and thickness, and it is likely that xylem in the midrib and
293 higher-order veins would collapse, especially as the Arabidopsis xylem cell walls are helicoidal,
294 and mostly consisting of thick primary walls (Figure 8). Future studies are needed to investigate
295 the collapse of xylem and its influence on the rehydration capacity of strongly dehydrated leaves.

296

297 *Response of leaf hydraulic conductance to dehydration and coordination with gas-exchange*

298 In Arabidopsis, we did not observe any embolism in leaf xylem conduits prior to, or even
299 moderately past the point of stomatal closure and turgor loss point. This finding is consistent with
300 recent work on tomato and grapevine showing stomata closed before any embolism were observed
301 using an optical visualization technique (Hochberg et al., 2017; Skelton et al., 2017). Here, we
302 confirm this finding for the first time using microCT on Arabidopsis. This finding is also consistent

303 with a growing body of literature showing that typically no xylem embolism is observed prior to
304 turgor loss point (Charra-Vaskou et al., 2012; Delzon and Cochard, 2014; Bouche et al., 2016;
305 Brodribb et al., 2016b; Scoffoni et al., 2017a; Scoffoni et al., 2017b). A recent study on sunflower
306 showed that xylem embolism occurs after turgor loss point even in plants that acclimate to drought:
307 plants grown under water-limited conditions adjusted osmotically and had a more negative turgor
308 loss point (-0.3 MPa shift), and leaf xylem P_{50} also shifted to a more negative value (-0.6 MPa
309 shift) (Cardoso et al., 2018).

310 The diurnal variation observed in stomatal conductance (g_s) and net photosynthetic rate per
311 leaf area (A_{area}) was strongly driven by leaf water status, i.e., Ψ_{leaf} , as shown by our model-fitting
312 analyses. Further, our analyses indicated that the dynamics of Ψ_{leaf} , and thus of g_s and A_{area} were
313 strongly driven by the dehydration-induced decline of K_{leaf} , in turn, resulting from changes in
314 outside-xylem pathways. Thus, we found that in *Arabidopsis*, K_{leaf} declines more rapidly than g_s
315 with dehydration, increasing the ratio of g_s/K_{leaf} , such that transpiration would amplify the decline
316 in Ψ_{leaf} , and consequently that of g_s . Indeed, 40-65% of g_s decline was attributable to K_{leaf} decline,
317 for leaves dehydrated to less than 50% of stomatal closure. For more strongly dehydrated leaves,
318 given their reduced stomatal conductance, the transpiration-driven amplification of Ψ_{leaf} and g_s
319 decline by K_{leaf} vulnerability are small, and declining Ψ_{leaf} due to low soil water potential and/or
320 exogenous signals such as ABA or sugar production would be responsible for driving stomata to
321 full closure. The direct mechanisms for stomatal closure with declining Ψ_{leaf} require further
322 research. While most proximally, stomatal closure relates to solute transfer from guard cells to
323 pavement cells, this could be driven by declining cell volume, turgor, osmotic concentration or
324 water potential, in the epidermis and/or mesophyll, partially or fully mediated by ABA
325 accumulation, which in turn may be associated with declining cell volume and K_{leaf} decline in
326 dehydrating leaves (McAdam and Brodribb, 2016; Susmilch et al., 2017; Sack et al., 2018).
327 Indeed, ABA signaling may contribute to stomatal closure both directly at the guard cells and also
328 by contributing to K_{leaf} decline in dehydrating leaves by reducing cell membrane permeability in
329 the bundle sheath and mesophyll via changes in aquaporin expression (Shatil-Cohen et al., 2011;
330 Pantin et al., 2013). Indeed, our MOFLO 2.0 simulations showed that K_{ox} decline was best
331 explained by reduced cell membrane permeability and to a lesser extent, cell connectivity. In
332 *Arabidopsis*, stress-induced changes in cell membrane permeability, mediated by aquaporins, can
333 have a strong impact on root hydraulic conductance (Javot and Maurel, 2002).

334 Alternatively, some studies have suggested that photosynthetic rate and carbon sink
335 activities could regulate stomata and plant hydraulics (Nikinmaa et al., 2013; Körner, 2015;
336 Rockwell et al., 2018). Indeed, this is well known in cropping systems such as grapevine, where
337 the presence of strong sinks such as fruits have been shown to stimulate photosynthesis (Hofäcker,
338 1978; Petrie et al., 2000). Recent studies have found that excess cellular sugar concentrations under
339 high irradiance, and/or during dehydration could trigger stomata closure (Nikinmaa et al., 2013;
340 Rockwell et al., 2018). Excess sucrose may be transported to the guard cells by the transpiration
341 stream, and the subsequent increase in osmolytes at the guard cell apoplast could induce stomatal
342 closure in some species, especially during periods of high photosynthetic rates (Lu et al., 1995; Lu
343 et al., 1997; Kang et al., 2007a; Kang et al., 2007b). Indeed, the increase in sucrose concentration
344 at the guard cells could act as more than a simple osmolyte, as it can depolarize the guard cell
345 plasma membrane, activating potassium channels (Jarzyniak and Jasiński, 2014), and an increase
346 in the level of sugar-sensing enzymes in the guard cells can accelerate stomatal closure by
347 stimulating ABA production (Kelly et al., 2013; Van Houtte et al., 2013; Li et al., 2016; Li et al.,
348 2018; Medeiros et al., 2018). Additionally, excess sucrose concentrations can decrease K_{ox} and
349 thus K_{leaf} , potentially via deactivation of aquaporins (Kelly et al., 2017).

350 In conclusion, K_{leaf} , g_s and A_{area} show a coordinated decline during leaf dehydration in
351 *Arabidopsis*, with a potentially strong direct effect of declining K_{leaf} in inducing stomatal closure
352 via a decrease in water potential. The decline in K_{leaf} and stomatal conductance may be jointly
353 driven by accumulation of sugar and/or ABA accumulation in dehydrating leaves, or K_{leaf} declines
354 may contribute to this accumulation. Future studies are needed to decipher the exact sequence of
355 events leading stomata to close.

356

357

358 *Putative role of K_{ox} decline in improving plant carbon balance, water-use efficiency and survival*
359 *during drought*

360 Why would the water transport pathways outside the xylem decline in efficiency during
361 dehydration prior to turgor loss point if this reduces gas exchange? Results from SurEau
362 simulations indicated that a vulnerable K_{ox} (and thus K_{leaf}) above turgor loss point leads to greater
363 water-use efficiency, and cumulative CO_2 assimilation, as well as protection of xylem from
364 embolism, and increased plant survival during drought. Simulated plants with K_{ox} declining prior
365 to turgor loss point operated on average at a lower K_{ox} value than plants with a K_{ox} held constant

366 (set to the average value measured at Ψ_{leaf} of -0.1 to -0.2 MPa, i.e., the range at which g_s was at its
367 maximum). This dynamic K_{ox} with water potential caused an up to 60% decline in g_s but only an
368 up to 12% decline in CO_2 assimilation, resulting in a higher water-use efficiency and greater
369 overall assimilation when considered over the entire period of soil drying. This benefit for low K_{ox}
370 raises the question of why plants should invest in a high K_{ox} (or K_{leaf}) in maximally hydrated leaves.
371 Indeed, high K_{leaf} values at Ψ_{leaf} above -1.0 MPa have at times been neglected when constructing
372 vulnerability curves (Blackman et al., 2012; Blackman et al., 2014) under the presumption that
373 leaves simply do not operate at such high water potentials in planta. However, a high K_{ox} (and
374 thus K_{leaf}) in well-hydrated leaves which declines during dehydration prior to turgor loss point
375 would offer advantages; it would enable high g_s and greater CO_2 assimilation under well-watered
376 conditions. This would be particularly beneficial for a short-lived species such as Arabidopsis,
377 which is required to grow rapidly when water availability is high. Previous studies have found that
378 maximum K_{ox} (and K_{leaf}) was high and declined rapidly with water potential in herbs (Scoffoni et
379 al., 2011; Nolf et al., 2016; Scoffoni et al., 2017a) than long-lived drought tolerant chaparral trees
380 (Scoffoni et al., 2017a). Ephemeral species such as Arabidopsis, or desert plants with short-lived
381 leaves, would especially benefit from high CO_2 assimilation rates, and thus high K_{leaf} after a rainfall
382 event, and “gear down” by reducing K_{leaf} and thus transpiration rates when water becomes scarce,
383 to improve their water-use efficiency and survive longer under soil drying (Grubb, 1998). In these
384 simulations, root hydraulic vulnerability had a small effect on water-use efficiency in Arabidopsis.
385 The much greater effect of leaf over root is due to the very high proportion of hydraulic resistance
386 in the leaf (85.7%), due to the lack of stem in the vegetative phase of this rosette species. The
387 hydraulic vulnerability of roots and their influence on the control of gas exchange is still under
388 debate given experimental challenges. Debate is ongoing over whether root xylem is highly
389 vulnerable (Hacke and Sauter, 1996; Hacke et al., 2000), or resistant (Rodriguez-Dominguez et
390 al., 2018) to xylem embolism. However, just as in leaves (Scoffoni et al., 2017a), the root extra-
391 xylem flow pathways might be more vulnerable. In grapevine, lacunae formation in fine root
392 cortical cells may cause a strong decline in K_{root} under drying-soil conditions, which would help
393 decouple the plant from drying soil and preserve its vascular system from embolism (Cuneo et al.,
394 2016). Notably, plant competition for soil water is not simulated in the SurEau model. As such,
395 we assume that plants have evolved to efficiently utilize soil water, and not overspend it (Cowan,
396 1982; Buckley et al., 2017b).

397

398 *The light response of leaf hydraulic conductance in Arabidopsis*

399 Maximum K_{leaf} for well-hydrated leaves often increases in response to light; this response has been
400 found for 15 of 30 species tested, in species of 23 plant families (Sack et al., 2002; Gasco et al.,
401 2004; Tyree et al., 2005; Cochard et al., 2007; Scoffoni et al., 2008; Voicu et al., 2008; Guyot et
402 al., 2012; Xiong et al., 2018). Further, in some species, the light enhancement of K_{leaf} is reduced
403 in dehydrated leaves, or, equivalently for those species, K_{leaf} declines with dehydration more
404 steeply under high irradiance (Guyot et al., 2012). In *Arabidopsis*, a previous study suggested that
405 the hydraulic conductance of entire rosettes of *Arabidopsis* had increased when acclimated to low
406 rather than high irradiance (Prado et al., 2013). In our experiments using the evaporative flux
407 method, we found significantly higher K_{leaf} values throughout the range of water potentials tested
408 for leaves acclimated to high irradiance, with a 60% enhancement of K_{leaf} from low to high
409 irradiance in well-hydrated leaves of Col-0. Discrepancies between these results may have arisen
410 due to methodological differences, given that in the study of Prado et al. (2013), hydraulic
411 conductance was measured by pushing water inward through the stomata of entire rosettes
412 suspended under water in darkness within a pressure chamber.

413 Notably, the light enhancement in K_{leaf} found in *Arabidopsis* did not result in a shift in P_{50} .
414 This finding indicates a proportional shift to lower values under low irradiance, throughout the
415 range of water potentials, contrary to findings for four woody species in which leaves acclimated
416 to high irradiance were more vulnerable to K_{leaf} decline with dehydration (Guyot et al. 2012). The
417 light enhancement of K_{leaf} would provide a greater hydraulic supply to meet the demand of leaves
418 acclimated to high irradiance, i.e., given strong and rapid dynamics of air temperature and
419 humidity and wind, and thus higher vapor pressure deficit and leaf boundary layer conductance.
420 Further, given strong transient dehydration during transpiration under these conditions, the higher
421 K_{leaf} would contribute to rapid mesophyll rehydration at high water potential and thus enable the
422 recovery of g_s and photosynthetic rate. The light enhancement of K_{leaf} could be caused by stronger
423 temperature gradients throughout the leaf under high light and/or changes in aquaporin expression
424 (Cochard et al., 2007). Our simulations of K_{ox} under low and high light using MOFLO 2.0 indicated
425 that K_{leaf} would be minimally enhanced by temperature gradients in the leaf caused by light
426 absorption, pointing to a role for aquaporins instead. This is consistent with the molecular evidence
427 that aquaporin expression is sensitive to light (Cochard et al., 2007; Ben Baaziz et al., 2012) and

428 that multiple aquaporin isoforms are involved in a range of responses such as K_{leaf} decline during
429 drought and K_{leaf} light enhancement (Cochard et al., 2007; Pou et al., 2013; Laur and Hacke, 2014b,
430 a). Furthermore, aquaporins may also be involved in cell rehydration (Marco et al., 2016). Finally,
431 aquaporins have also been suggested to play a role in a rapid enhancement of K_{leaf} when
432 *Arabidopsis* is suddenly exposed to low relative humidity, compensating for the increased
433 evapotranspiration, and allowing stomata to remain open (Levin et al., 2007).

434

435 *Contribution of hydraulic traits to Arabidopsis thaliana whole-plant physiology*

436 *Arabidopsis* Col-0 has high values of K_{leaf} , g_s and A_{area} relative to previously published values of
437 diverse angiosperm species (Flexas et al., 2013; Scoffoni and Sack, 2017), and displays strong
438 sensitivity in K_{leaf} and gas exchange to dehydration. This physiological behavior is consistent with
439 *Arabidopsis*' ruderal ecology, establishing and producing flowers and seeds in open or disturbed
440 habitats in spring/early summer (Koornneef et al., 2004). The high values of K_{leaf} were driven by
441 an especially high K_{ox} ($106 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$). This high K_{ox} is not untypical in herbs; in *Salvia*
442 *canariensis*, maximum K_{ox} reached $231 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ (Scoffoni et al., 2017a). Notably, a
443 high K_x , K_{ox} and K_{leaf} are often achieved with allocation to substantial vein length per area (VLA),
444 which increases flow paths in parallel within the xylem and reduces flow distance outside the
445 xylem (Sack and Scoffoni, 2013); *Arabidopsis* possesses a relatively low VLA but flow distance
446 is strongly reduced by its very thin leaf, which would also reduce K_{ox} (Brodribb et al., 2007;
447 Buckley et al., 2015). Furthermore, a high aquaporin activity, and/or cell wall permeability
448 especially at the bundle sheath could potentially influence K_{ox} ; across several *Arabidopsis* mutants,
449 maximum K_{leaf} was associated with an anatomical index of bundle sheath conductivity (Caringella
450 et al., 2015). The high K_x value could potentially arise from xylem structure, i.e., the numbers and
451 sizes of xylem cells within minor veins (Caringella et al., 2015; Stewart et al., 2018), in
452 combination with high conductance between xylem conduits. Indeed, our TEM imaging showed
453 very little secondary lignification of xylem conduits throughout the midrib and other vein orders
454 (Figure 8), such that the bulk of midrib conduit walls are effectively one large pit membrane (i.e.,
455 primary unligified wall) with water potentially leaking throughout the surface, a structure that
456 would strongly reduce pit wall resistance and thus total xylem resistance (Choat et al., 2008).

457 *Arabidopsis* Col-0 also exhibits strong drought sensitivity, with its very low leaf mass per
458 area (Wright et al., 2004), a very high degree of area shrinkage during dehydration (58% shrinkage

459 when dry), high minimum epidermal conductance (g_{\min}), very high osmotic potential at full turgor,
460 low modulus of elasticity and relative water content at turgor loss point, and a turgor loss point
461 that is among the highest values reported across angiosperm species (Bartlett et al., 2012), similar
462 to that of the water potential at stomatal closure (g_{s95}) and at 88% loss of K_{leaf} , around -0.7MPa.
463 This detailed characterization of *Arabidopsis* Col-0 hydraulics traits, and their dynamics during
464 leaf dehydration and implications for whole-plant responses highlights useful avenues for high
465 throughput phenotyping, and the elucidation of genetic mechanisms controlling these key traits,
466 which would be loci for manipulation of gas exchange and drought tolerance.

467

468 **Material and methods**

469 *Plant material and growth conditions*

470 Measurements were performed on *Arabidopsis thaliana* Col-0 (ecotype Colombia), hereafter
471 referred to as *Arabidopsis*, grown continuously from December 2015 through November 2016. We
472 grew *Arabidopsis thaliana* in a climate-controlled greenhouse at the University of California, Los
473 Angeles. Seeds were sown in lawns in pots (3.13” width x 4.88”length x 2.31” deep) in soil
474 (1:1:2:1:1 mixture of washed plaster sand, loam, peat moss, perlite, vermiculite), and cold-
475 acclimated at 4°C for three days in a chamber, then brought to the temperature-controlled
476 greenhouse (minimum, mean, and maximum values for temperature, 19.3°C, 22.5°C, and 33.2°C;
477 for humidity, 24%, 63%, and 92%; for irradiance (from 0900 to 1600), 11.2, 169, 1369 μmol
478 $\text{photons m}^{-2} \text{s}^{-1}$). We recognize that many researchers often grow *Arabidopsis* in growth chambers
479 under $<300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance, and future work should consider the variation in leaf
480 physiology, morphology and anatomy driven by this lower irradiance. We chose to grow
481 *Arabidopsis* in a greenhouse setting where plants are exposed to light fluctuations, with temporary
482 high light peaks, as is experienced in the field. Indeed, this has been shown to impact plant growth
483 (Poorter et al., 2016). Further, growing plants under such high irradiance means that these were
484 not light-limited, and thus, our findings can be compared with those for other species grown
485 without light limitation, as is typical in studies of plant hydraulic physiology.

486 When plants had true leaves after approximately one week, they were thinned to one
487 individual per pot. Plants were watered regularly to keep soil moist. After approx. 6 weeks, at
488 which points plants had >10-20 leaves, mature and healthy leaves were chosen for gas exchange,
489 hydraulic and x-ray micro-computed tomography measurements.

490

491 *Leaf hydraulic conductance*

492 Pots were transported to the laboratory, watered and enclosed overnight in plastic bags filled with
493 wet paper towels to ensure a saturated atmosphere. To obtain a vulnerability curve spanning a
494 range of leaf water potential (Ψ_{leaf}), well-hydrated and dehydrated leaves were measured. To obtain
495 K_{leaf} values at high Ψ_{leaf} , mature and healthy leaves were directly cut at their base under water and
496 their petioles placed in a petri dish containing ultra-pure water (0.22 μm Thornton 200 CR;
497 Millipore) prior to being connected to the evaporative flux system described below. To obtain K_{leaf}
498 values at low Ψ_{leaf} , individuals were removed from the soil and dehydrated on the bench for 0.25-
499 2 hours, after which they were placed in bags which had previously been exhaled into, within a
500 second bag filled with wet paper towels, to ensure high vapor and CO_2 concentration, to reduce
501 stomatal opening and facilitate equilibration for 30 min. Two leaves were then measured for initial
502 Ψ_{leaf} , using a pressure chamber (Plant Moisture Stress Model 1000; PMS Instrument Co, Albany,
503 OR, USA), with a grass fitting in the compression lid; for a few leaves with round petioles, silicon
504 adaptors were used (Shatil-Cohen et al., 2011). On average, the two leaves measured for initial
505 Ψ_{leaf} differed by $0.051 \text{ MPa} \pm 0.008$ standard error. A third mature and healthy leaf from the
506 dehydrated individual was measured for K_{leaf} . After the leaf petiole was cut under water, it was
507 gently wrapped with parafilm and connected via tubing to a water source on a balance ($\pm 10 \mu\text{g}$,
508 models XS205 and AB265; Mettler Toledo, Columbus, OH, USA), which logged the flow rate
509 into the leaf every 5 s to a computer. The leaf was placed over a fan and under a light source
510 ($>1000 \mu\text{mol m}^{-2}\text{s}^{-1}$; model 73828, 1000 W UV filter; SearsRoebuck, Hoffman Estates, IL, USA).
511 A water bath was placed between the leaf and the light to avoid overheating the leaf, which was
512 kept between 23 and 28°C as measured using a thermocouple (Cole-Parmer). After a minimum of
513 30 min to ensure light acclimation (Scoffoni et al., 2008) and once the flow had stabilized with no
514 upward or downward trend, the average steady state flow rate for the last 5 min was recorded and
515 leaf temperature was measured (Cole-Parmer). The leaf was rapidly removed from the system, its
516 petiole dabbed dry, and placed in a bag which had previously been exhaled into. The bagged leaf
517 was placed into a second bag filled with wet paper towels, and left to equilibrate for 30 min, after
518 which final Ψ_{leaf} was measured. Leaf area was manually traced onto paper, scanned, and measured
519 using ImageJ software (version 1.46r; National Institutes of Health). Leaf hydraulic conductance
520 (K_{leaf}) was calculated as the flow rate divided by the leaf water potential driving force (the water

521 potential of the water fed to the petiole [0 MPa] minus measured Ψ_{leaf} , normalized by leaf area
522 and corrected for the dependence of water viscosity on temperature (to a reference value of 25°C;
523 Weast, 1974 ; Yang and Tyree, 1993); this correction also approximately applies for the
524 temperature dependence of vapor phase transport across this range of temperature (Buckley, 2015).
525 Leaf hydraulic vulnerability curves were obtained as the plot of K_{leaf} vs. the most negative Ψ_{leaf}
526 experienced by the leaf (either the initial or final).

527 K_{leaf} vulnerability curves were measured under very low laboratory irradiance (light source
528 off; $<3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high irradiance ($>1000 \mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements in very
529 low and high irradiance were performed on the same day using leaves taken from the same
530 individuals when possible, i.e., when two leaves from the same individual were mature and healthy.
531 Notably, the aim of this experiment was to test for a rapid light enhancement of K_{leaf} for high-
532 light-grown individuals. Future studies are needed to investigate the plasticity in K_{leaf} and other
533 physiological and morphological traits for *Arabidopsis* across different light growth regimes, as
534 found to be important in a study of species of Hawaiian lobeliads (Scoffoni et al., 2015).

535

536 *Leaf xylem hydraulic conductance*

537 Leaf xylem hydraulic conductance was measured for six leaves (taken from six different
538 individuals) using the vacuum pump method (Kolb et al., 1996; Nardini et al., 2001; Sack et al.,
539 2004; Scoffoni and Sack, 2015; Trifilo et al., 2016). Briefly, individuals were rehydrated in the
540 laboratory overnight, and kept in dark plastic bags filled with wet paper towels to ensure high
541 humidity. The next morning, a leaf was cut off the plant under ultra-pure water and placed in a
542 petri dish over a white light transilluminator table (Model TW, UVP, Upland, CA, USA) to allow
543 visualization of the fourth-order veins (=minor veins). Using a fresh scalpel, 8-15 cuts per cm^2 of
544 leaf area were made to the lamina, severing minor veins, to ensure that outside-xylem pathways
545 would be bypassed (Sack et al., 2004; Nardini and Salleo, 2005; Nardini et al., 2005b; Sack et al.,
546 2005). Great care was taken to avoid cutting major veins; if they were cut by accident, the leaf was
547 discarded. Once the cuts were made, the leaf petiole was wrapped with parafilm and inserted
548 through a small rubber stopper that had been perforated using a cork borer. The small rubber
549 stopper was then connected to a tube fitting connected to silicone tubing (ColeParmer, Vernon
550 Hills, IL, USA). The rubber stopper allowed a good seal around the petiole without crushing. We
551 obtained a vacuum tight seal by tightening the tubing around the rubber stopper with zipties and

552 sealing the petiole to the exposed end of the rubber stopper using super glue (Loctite 409 glue;
 553 Henkel Corp., Los Angeles, CA, USA) with accelerator (Loctite 712 accelerator). Leaves were
 554 placed inside a vacuum flask with a thermocouple (Cole-Parmer) connected by a fourway valve to
 555 a vacuum pump (Gast) and a high-precision pressure gauge (± 0.002 MPa; J4605 Marsh/Bellofram;
 556 Marshall Instruments Inc., Anaheim, CA, USA).

557 We applied five increasing levels of partial vacuum, resulting in absolute pressures
 558 between 0.06 and 0.02 MPa, and recorded the flow rate of water entering the leaf from a water
 559 source on a balance (± 10 μg , models XS205 and AB265; Mettler Toledo, Columbus, OH, USA).
 560 The average flow rate of the last 5 min of stability for a given pressure was recorded, along with
 561 the temperature. The flow rate was normalized to 25°C correct for the temperature response of the
 562 viscosity of water (Weast, 1974 ; Yang and Tyree, 1993). Leaf xylem hydraulic conductance (K_x)
 563 was calculated as the slope of the flow rate vs. pressure, and normalised by leaf area, measured at
 564 the end of the experiment with a flatbed scanner. The percent hydraulic resistance in the leaf xylem
 565 ($\%R_x$) and outside-xylem ($\%R_{ox}$) were calculated as:

566

$$567 \quad (1) \quad \%R_x = \frac{1/K_x}{1/K_{\text{leaf}}} \times 100$$

$$568 \quad (2) \quad \%R_{ox} = 100 - \%R_x$$

569

570 *Diurnal measurements of stomatal conductance, photosynthetic rate and whole plant hydraulic*
 571 *conductance as a function of leaf water potential*

572 Diurnal measurements of light-saturated photosynthetic rate (A_{max}) and stomatal conductance (g_s)
 573 were performed in the greenhouse on 40 individuals on 10-11 November 2016 from 0900 to 1800
 574 using a portable gas exchange system (LI-6400; LI-Cor, Lincoln, NE, USA). The chamber CO_2
 575 was set at 400 ppm and irradiance at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, and
 576 leaf to air vapor pressure deficit was maintained between 0.4 and 0.6 kPa. Measurements were
 577 taken after the leaf had equilibrated in the chamber for 10 min; A_{max} and g_s were logged 5 times at
 578 10-sec intervals, and these five measurements were averaged. We checked that 10 min was
 579 sufficient equilibration time; $n = 7$ leaves were kept in the chamber for an additional five minutes;
 580 no significant differences were found between values taken at 10 vs. those taken at 15 min (paired
 581 t -test; $p = 0.08$). To verify g_s measurements, additional measurements were taken using a

582 porometer on the abaxial side of the leaf (Delta-T Devices; Cambridge, UK) on 10-11 November
 583 2016 from 0900 to 1800. As expected, the g_s values obtained from the LICOR and porometer were
 584 within the same range of values and thus were pooled together during the analyses.

585 At the end of the measurement, the leaf was excised with a razor blade and immediately
 586 placed in a sealable bag (Whirl-Pak, Nasco, Fort Atkinson, WI, USA), which had been previously
 587 exhaled in, and the bagged leaves were placed in a second bag filled with wet paper towels. After
 588 at least 30-min equilibration, Ψ_{leaf} was measured using a pressure chamber as described above.

589 Whole-plant hydraulic conductance (K_{plant} ; $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was estimated under the
 590 assumption that soil water potential was fully saturated throughout the day (thus, $\Psi_{\text{soil}} = 0 \text{ MPa}$).
 591 Though we did not directly measure Ψ_{soil} , plants were well-watered and soil was always moist.
 592 Thus, K_{plant} was determined from the stomatal conductance obtained from the porometer data
 593 described above (measurements performed under ambient light irradiance), ambient VPD at the
 594 time of measurement, and leaf water potential:

595
 596 (3)
$$K_{\text{plant}} = \frac{g_s \times \text{VPD}}{\Psi_{\text{soil}} - \Psi_{\text{leaf}}}$$

599 *X-ray micro-computed tomography*

600 To visualize leaf vein xylem embolism and tissue shrinkage, we used X-ray micro-computed
 601 tomography (microCT) at the synchrotron at the Advanced Light Source (ALS) in Berkeley,
 602 California (Beamline 8.3.2). We imaged the xylem within the midrib and lamina tissues in 18
 603 leaves of a range of leaf water potential from 9 individuals in February 2016 at 1.27- μm resolution.
 604 Three additional individuals were further scanned in November 2016 at a higher resolution of
 605 0.638 μm to check for potential collapse in xylem conduits of the midrib. Arabidopsis individuals
 606 grown as described above were transported as carry-on in a plane to ALS. Individuals were fully
 607 rehydrated at the start of the experiment, and whole plants were removed from the soil and
 608 dehydrated on the bench for different times to obtain a range of water potentials, and equilibrated
 609 in double-sealed plastic bags for 30 min, after which two leaves were excised to obtain initial leaf
 610 water potential. Two of the leaves remaining attached to the plant were juxtaposed within a
 611 styrofoam holder and 0.653-0.869 mm length scans were made of their midrib and surrounding
 612 lamina at the center of each leaf. A small piece of copper wire was attached at the center of the

613 leaves to help center the samples for scanning. Kapton tape (DuPont, Wilmington, DE, USA) was
614 used to tape the leaves and the copper wire to the styrofoam holder to minimize sample movement
615 during the scan. The styrofoam with the sample enclosed was placed in a Plexiglass cylinder,
616 attached to a custom-built aluminium sample holder mounted on an air-bearing stage, and wet
617 paper towels were placed above the sample in the Plexiglass cylinder to minimize evaporation
618 during the measurement. At the end of the measurement, final leaf water potential was recorded
619 and leaf areas were measured. No significant differences in water potential before and after the
620 measurement were observed (paired *t*-test; $p = 0.70$; $n = 8$). Scans were made at 20-23 keV in the
621 synchrotron X-ray beam, and rotated 180° with the instrument to enable visualization of the full
622 3D internal structure of the leaf. Scans took 5-10 min to complete depending on the scan area
623 selected. Three-dimensional volume renderings were made using the AVIZO 8.1.1 software (VSG
624 Inc., Burlington, MA, USA), and used to count the number of embolized conduits in the entire
625 sample and different vein orders. For the four samples that showed embolism, we measured the
626 length of the embolized conduit and the widths of both conduit axes at three locations along the
627 sample length. We also visualized for each section the water-filled conduits within the midrib and
628 secondary veins to observe any potential deformation or collapse.

629 Using ImageJ software (version 1.46r; National Institutes of Health), we measured lamina
630 tissue and cell dimensions on three cross-sectional images randomly selected in the middle of each
631 sample. For each image, we measured thickness of the lamina and of each tissue, i.e., the abaxial
632 and adaxial epidermises including the cuticle, and the palisade and spongy mesophyll, at three
633 locations within the sample. We also measured the area, perimeter and diameters as well as the %
634 intercellular airspace of palisade and spongy mesophyll cells.

635

636 *Drought tolerance traits*

637 The leaf turgor loss point, osmotic potential at full turgor, relative water content at turgor loss point
638 and modulus of elasticity were calculated from a pressure-volume curve constructed using 29
639 leaves from 20 individuals previously rehydrated overnight (Supplemental Figure S3). Initial leaf
640 mass was obtained for each single leaf before dehydration to a range of Ψ_{leaf} . Leaf water potentials
641 were measured with a pressure chamber after 30 min of equilibration in bags with high humidity,
642 after which the leaf mass was measured again, along with leaf area, before it was placed in a drying

643 oven at 70°C and measured for dry mass after 72 hours. Pressure-volume curve parameters were
644 obtained following standard protocols (Sack and PrometheusWiki, 2010).

645 We measured the minimum epidermal conductance (=cuticular plus residual stomatal
646 conductance; g_{\min}) on nine mature leaves from nine individuals in June 2016 by following a
647 standard protocol (Sack et al., 2010). Individual leaves were rehydrated covered in plastic in the
648 laboratory the night before measurements. The next day, nine leaves excised, their cut petioles
649 were sealed with wax, and their fresh mass and leaf area were measured, before dehydration for
650 an hour taped to a fishing line above a fan, to ensure stomatal closure. Leaves were then repeatedly
651 taken off the fan and bagged and measured for mass every 20 min. After eight measurements were
652 obtained for a given leaf, its area was measured again. The g_{\min} was calculated as the slope of mass
653 over time divided by the average mole fraction vapor pressure deficit (VPD) during the
654 measurement and normalized by the average of the initial and final leaf area given shrinkage with
655 dehydration during measurement. VPD was calculated from the temperature and relative humidity
656 measurements obtained from a weather station (HOBO Micro Station with Smart Sensors, Onset,
657 Bourne, MA, USA). Finally, each individual leaf was dried in an oven at 70°C for three days, and
658 dry mass and area were obtained to calculate leaf dry mass per hydrated area (LMA ; in g m^{-2}) and
659 the percent area shrinkage in the dried leaf relative to the hydrated leaf (PLA_{dry} ; %).

660

661 *Leaf anatomy*

662 Data for leaf venation and leaf cross-sectional anatomy of Col-0 to aid with interpretation of
663 microCT images were obtained from a previous study (Caringella et al., 2015).

664 To visualize xylem conduit walls, transmitted electron microscopy (TEM) was performed
665 on three leaves from three Col-0 individuals in Germany. Small samples (ca. 2 mm wide and 8
666 mm long) from leaf midribs (and surrounding mesophyll) were cut under water and fixed in
667 glutaraldehyde (2.5% glutaraldehyde, 0.1 mol phosphate, 1% saccharose, pH 7.3) overnight. After
668 being washed in phosphate buffer and post-fixed with 2% OsO₄, samples were dehydrated in a
669 series of propanol solutions (30%, 50%, 70%, 90% and three times 100%). Samples were finally
670 immersed in 1.2-propylenoxide (CAS-Nr. 75.56-9, Fontenay-sous-Bois, France) and gradually
671 embedded in Epon resin (Sigma-Aldrich, Steinhilber, Germany) and polymerized at 60°C for 48 h.
672 Ultra-thin sections (<90 nm thick) were made with a Leica Ultracut UTC microtome (Leica
673 Microsystems GmbH, Wetzlar, Germany) and placed on copper slot grids). Observations were

674 made using a JEOL 1400 TEM (JEOL, Tokyo, Japan) at an accelerating voltage of 120 kV. Images
675 were taken with a digital camera (Soft Imaging System, Münster, Germany).

676

677 *Modelling of hydraulic function across scales from tissues to whole plant*

678 We applied a framework of four models across scales to compute the mechanisms underlying K_{leaf}
679 decline inside and outside the xylem, the causal role of K_{leaf} decline in driving stomatal closure,
680 and the implications for gas exchange under simulated drought regimes (Table 1).

681 We first estimated the causal importance of mechanistic drivers of K_{leaf} decline using
682 spatially explicit models of the leaf veins (K_LEAF; Cochard et al., 2004b; Scoffoni et al., 2017b)
683 and outside-xylem pathways (MOFLO 2.0; Buckley et al., 2017a). Using K_LEAF, we tested
684 whether xylem embolism and/or conduit collapse could explain the observed decline in K_{leaf} . We
685 first tested for the impact of the embolisms observed with microCT imaging in the midrib and
686 secondary veins on the xylem hydraulic conductance (K_x) and ultimately K_{leaf} (see Supplemental
687 Methods for more information on model parameterization; Supplemental Table S2). We tested the
688 potential effect of the collapse of tertiary and minor vein conduits on K_x under two scenarios: (1)
689 a “realistic” impact of conduit collapse on conduit conductivity (13% decline in tertiary and minor
690 vein conductivity, similar to that observed in *Quercus rubra* at turgor loss point by Zhang et al.,
691 2016), and (2) a more severe conduit collapse scenario which would induce 50% decline in tertiary
692 and minor vein conduit conductivity (see Supplemental Methods). Using MOFLO 2.0, we
693 investigated the potential drivers of decline in outside-xylem hydraulic conductance with
694 dehydration. We simulated the impact of an 80% decline in cell membrane permeability, and/or
695 decline in cell-to-cell liquid phase hydraulic connectivity given the anatomical changes due to cell
696 shrinkage at -0.5 MPa under different scenarios: (1) with or without an apoplastic barrier to liquid-
697 phase water transport across the bundle sheath, (2) under either no light or with an irradiance of
698 $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation to clarify a potential role of transdermal
699 temperature gradients (Supplemental Methods; Supplemental Table S2).

700 We then quantified the direct influence of K_{leaf} decline on g_s decline with dehydration, using
701 a partitioning approach. We first considered the empirical maximum likelihood functions relating
702 K_{leaf} and g_s to leaf water potential:

703

704 (4) $g_s = -451 \times |\Psi_{\text{leaf}}| + 339$

705

706 (5) $K_{\text{leaf}} = 6.83 + 81.4 \exp(-7.56 \times |\Psi_{\text{leaf}}|)$

707

708 Ψ_{leaf} is in turn a function of g_s , K_{leaf} , soil water potential (Ψ_{soil}) and the water vapor mole fraction
709 gradient (Δw):

710

711 (6) $\Psi_{\text{leaf}} = \Psi_{\text{soil}} - \Delta w \frac{g_s}{K_{\text{leaf}}}$

712

713 As Ψ_{leaf} declines during leaf dehydration, the resulting declines in g_s and K_{leaf} lead to changes in
714 their ratio, g_s/K_{leaf} . If K_{leaf} declines more rapidly than g_s with Ψ_{leaf} , such that the ratio g_s/K_{leaf}

715 increases, the decline in Ψ_{leaf} will be amplified, and consequently so will the decline in g_s itself.

716 Therefore, K_{leaf} decline with dehydration would contribute to stomatal closure. The fraction of g_s
717 decline with Ψ_{leaf} that can be attributed to K_{leaf} decline, F , is

718

719 (7) $F = \frac{\frac{\partial g_s}{\partial K_{\text{leaf}}} \frac{\partial K_{\text{leaf}}}{\partial \Psi_{\text{leaf}}}}{\frac{\partial g_s}{\partial \Psi_{\text{leaf}}}}$,

720

721 where the partial derivative in the numerator is the sensitivity of g_s to Ψ_{leaf} with Ψ_{soil} and Δw held
722 constant (1.5 kPa). That partial derivative is given by

723

724 (8) $\frac{\partial g_s}{\partial K_{\text{leaf}}} = \frac{\partial g_s}{\partial \Psi_{\text{leaf}}} \frac{\partial \Psi_{\text{leaf}}}{\partial K_{\text{leaf}}} = \frac{\partial g_s}{\partial \Psi_{\text{leaf}}} \left[-\Delta w \left(\frac{1}{K_{\text{leaf}}} \frac{\partial g_s}{\partial K_{\text{leaf}}} - \frac{g_s}{K_{\text{leaf}}^2} \right) \right]$

725

726 Solving for $\partial g_s / \partial K_{\text{leaf}}$ gives

727

728 (9) $\frac{\partial g_s}{\partial K_{\text{leaf}}} = \frac{\frac{\partial g_s}{\partial \Psi_{\text{leaf}}} \frac{g_s \Delta w}{K_{\text{leaf}}^2}}{1 + \frac{\partial g_s}{\partial \Psi_{\text{leaf}}} \frac{\Delta w}{K_{\text{leaf}}}} = \frac{\frac{\partial g_s}{\partial \Psi_{\text{leaf}}} \frac{g_s}{K_{\text{leaf}}}}{\frac{\partial g_s}{\partial \Psi_{\text{leaf}}} + \frac{K_{\text{leaf}}}{\Delta w}}$

729

730 Combining 7 and 9 gives F as

731

732 (10) $F = \frac{\frac{\partial K_{\text{leaf}}}{\partial \Psi_{\text{leaf}}} \frac{g_s}{K_{\text{leaf}}}}{\frac{\partial g_s}{\partial \Psi_{\text{leaf}}} + \frac{K_{\text{leaf}}}{\Delta w}}$

733

734 Finally, using a simplified discrete-time soil–plant hydraulic model (SurEau; Martin-
735 StPaul et al., 2017), we estimated the influence of K_{leaf} decline on stomatal closure under varying

736 simulations of soil and atmospheric drought. We simulated transpiration, stomatal conductance,
 737 cumulative photosynthetic rate, cumulative water-use efficiency, water potential and the percent
 738 loss of xylem hydraulic conductance (PLC) daily and during the course of soil drying until plant
 739 death (i.e., PLC = 100%). We performed these simulations following four different scenarios: (1)
 740 K_{leaf} and K_{root} were both vulnerable to dehydration prior to turgor loss point (using the function of
 741 K_{leaf} vs. Ψ_{leaf} measured with the EFM, and the vulnerability of K_{root} obtained from that of K_{leaf} and
 742 K_{plant} , assuming no stem resistance in Arabidopsis; Supplemental Figure S4); (2) K_{leaf} was
 743 vulnerable but not K_{root} (K_{root} was kept constant until xylem embolism occurred in the root); (3)
 744 K_{root} was vulnerable but not K_{leaf} (K_{leaf} was kept constant until xylem embolism occurred in the
 745 leaf); and (4) neither K_{leaf} nor K_{root} were vulnerable to dehydration (i.e., both K_{leaf} and K_{root} were
 746 kept at constant values until xylem embolism occurred) (Supplemental Methods; Supplemental
 747 Table S3).

748

749 *Statistics*

750 We selected functions for the responses of K_{plant} , K_{leaf} , g_s and A_{max} to Ψ_{leaf} using a maximum
 751 likelihood framework (Burnham and Anderson, 2002; Sack et al., 2006). For the g_s and A_{max} curve
 752 fitting, extremely low values at the beginning or end of the day when stomata were shut in well-
 753 hydrated leaves ($\Psi_{\text{leaf}} > -0.01$ MPa) were discarded, and likely represented the effects of the
 754 mechanical advantage of epidermal cells preventing stomatal opening in turgid leaves (Guyot et
 755 al., 2012); these points represented 3/63 and 2/26 of the points for g_s and A_{max} respectively. We
 756 selected the maximum likelihood model using the `optim` function in R 3.4.1 ([http://www.r-](http://www.r-project.org)
 757 [project.org](http://www.r-project.org);). We fitted four types of functions to the curves, as previously used in the literature
 758 (Scoffoni et al., 2012), where $Y = K_{\text{leaf}}$, A_{area} or g_s , and Ψ_{leaf} is leaf water potential: linear ($Y = a$
 759 $\Psi_{\text{leaf}} + y_0$), two-parameter sigmoidal ($Y = a / (1 + e^{-(\Psi_{\text{leaf}} - x_0)/b})$), logistic ($Y = a / (1 + (\Psi_{\text{leaf}} / x_0)^b)$),
 760 and exponential ($Y = y_0 + a \times e^{-(b \times \Psi_{\text{leaf}})}$). We estimated the maximum Y value by extrapolating to
 761 $\Psi_{\text{leaf}} = 0$ and, as indices of decline with dehydration, the Ψ_{leaf} at which maximum Y values
 762 decreased by 50% and 95%. Because the best-fit function for the K_{leaf} vulnerability curve was
 763 exponential and the Y value at $\Psi_{\text{leaf}} = 0$ was extrapolated to a very high unrealistic value, we also
 764 estimated the maximum K_{leaf} by averaging all points above -0.1 MPa (K_{max}), as has been typically
 765 done in the literature (i.e., Sack et al., 2003; Nardini et al., 2005a; Brodrribb and Jordan, 2008;
 766 Scoffoni et al., 2008; Scoffoni et al., 2015).

767 To test for an effect of light on K_{leaf} , we selected the best-fit function for the response of
 768 K_{leaf} to Ψ_{leaf} , combining data for laboratory irradiance and high irradiance treatments, using a
 769 maximum-likelihood framework as explained above. We then calculated the residual variation for
 770 each leaf, subtracting the measured K_{leaf} (and irradiance) from the predicted K_{leaf} at the given Ψ_{leaf} ,
 771 based on the best fit. We then performed a *t*-test on the residuals obtained for the high vs. low
 772 irradiance leaves across the entire range of Ψ_{leaf} , as well as just for points above turgor loss point,
 773 and for well-hydrated leaves (above -0.2MPa).

774 To determine the contribution of each correlated predictor variables (Time, PAR,
 775 Temperature, VPD, Ψ_{leaf}) to the observed variation in g_s diurnally, we applied independent effects
 776 analysis (Murray and Conner, 2009) using the hier.part function in R.3.4.1.

777

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786

787 Figure captions

788 **Figure 1.** Decline of leaf hydraulic conductance (K_{leaf}) measured under high ($>1000 \mu\text{mol}$
 789 photons $\text{m}^{-2} \text{s}^{-1}$) or low ($< 3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) irradiance. The maximum likelihood function
 790 is shown for K_{leaf} vulnerability acclimated under high light ($K_{\text{leaf}} = 6.83 + 81.4 \exp(-7.56 \times$
 791 $|\Psi_{\text{leaf}}|)$), and low light ($K_{\text{leaf}} = 8.98 + 84.2 \exp(-13.2 \times |\Psi_{\text{leaf}}|)$). The dashed line
 792 represents the water potential at 50% loss of K_{leaf} (similar in both treatments).

793

794 **Figure 2.** Plant hydraulic and gas exchange response to dehydration in Arabidopsis. Decline of
 795 the whole plant hydraulic conductance (K_{plant} ; A), stomatal conductance (g_s ; B) and light-
 796 saturated photosynthetic rate (A_{area} ; C) with dehydration. Each point represents a different
 797 measured leaf. K_{plant} was obtained from the porometer data by dividing transpiration by leaf

798 water potential (assuming soil water potential was at full saturation). The black fitted line in each
 799 pannel is the maximum likelihood function (exponential for $K_{\text{plant}} = 2.0 + 91.1 \exp(-7.75 \times$
 800 $|\Psi_{\text{leaf}}|)$; linear for $g_s = 339 - 451 \times |\Psi_{\text{leaf}}|$, and $A_{\text{area}} = 14.4 - 19.2 \times |\Psi_{\text{leaf}}|$). The dotted
 801 grey line is the leaf water potential (Ψ_{leaf}) at 50% loss of maximum K_{plant} , g_s or A_{area} . Because
 802 trait values above -0.1 MPa were especially low (white circles), likely representing stomatal
 803 closure at those high water potentials (see *Methods*), we did not include these points in the line
 804 fitting.

805

806 **Figure 3.** Lack of embolism observed in midrib conduits of *Arabidopsis thaliana* (Col-0) across
 807 levels of dehydration as revealed by in vivo images of leaf midribs subjected to progressive
 808 dehydration using micro-computed tomography (A-C). Water-filled cells appear in light grey in
 809 microCT. If air-filled (i.e., embolized) conduits were present, they would appear as black in the
 810 xylem portion of the midrib. There was no embolism, as shown in these images by the red
 811 arrows pointing at the entirely light grey midrib xylem. The leaf water potential (Ψ_{leaf}) has been
 812 provided for each image. The inset in (A) represents a leaf midrib cross-section imaged under
 813 light microscopy, with the red arrow pointing to the xylem tissue (dark blue conduits).

814

815 **Figure 4.** Rare embolisms were observed in a few individual leaves. In two samples, an
 816 embolized conduit was observed in the midrib; it continued into a secondary vein (A, F; the
 817 embolized conduits are depicted in yellow). The embolized conduit in the midrib and secondary
 818 vein can be seen in cross-sections (B, D, G, I) and longitudinal sections of the microCT scan (C,
 819 E, H, J). The arrows point to the embolized conduit (appearing as black in the microCT image).
 820 Because of the two dimensionalities of these sections, embolism in the midrib and secondary
 821 vein might appear disconnected (C, E). Note that while the embolism was present in only one
 822 conduit per cross-sectional image, multiple conduits spanned the length of the midrib and
 823 secondary vein (as can be observed in K, where two conduits can be seen connected to one
 824 another). Most likely, a first conduit in the midrib embolized, and all the conduits directly
 825 connected to that one upstream embolized after. In one sample, an embolized conduit was
 826 observed isolated in the secondary vein (L-N), while in another sample, an embolized conduit
 827 was observed spanning the midrib length (O-Q).

828

829 **Figure 5.** Results from simulations using a spatially explicit model of leaf outside-xylem water
 830 to test for potential drivers of the decline in K_{ox} in dehydrating leaves transport (MOFLO 2.0, see
 831 Table 1 and *Methods*). The K_{ox} was first computed based on the decline of observed cell size and
 832 air space alone (grey bars), which resulted in an increase in K_{ox} (negative percent loss of K_{ox} ;
 833 mainly due to shortening of pathways from the veins to stomata). We then modelled K_{ox} decline
 834 according to three scenarios (though always including the effect of tissue dimensional changes):
 835 an 80% decline at -0.5MPa in (1) cell connectivity (red bars), (2) cell membrane permeability
 836 (blue bars), and (3) cell wall thickness (black bars). All simulations were run with (Ap; darker
 837 color) or without an apoplastic barrier (No Ap; lighter color) at the bundle sheath cells. The
 838 yellow star on the x -axis represents the % observed K_{leaf} decline at -0.5MPa (measured with the
 839 evaporative flux method, see *Methods*).

840

841 **Figure 6.** Model simulations mapping the contribution of the decline of leaf hydraulic
 842 conductance (K_{leaf}) decline to that of stomatal conductance (g_s) with dehydration (Table 1).

843

844 **Figure 7.** Daily simulated patterns of stomatal conductance (A), leaf water potential (B),
 845 cumulative CO₂ assimilation (C) and the percent loss of leaf xylem hydraulic conductance (D)
 846 during the progression of a simulated soil drought (SurEau Model, see Table 1 and *Methods*).
 847 Four scenarios were modelled: (1) both leaf hydraulic conductance (K_{leaf}) and root hydraulic
 848 conductance (K_{root}) were vulnerable to dehydration prior to turgor loss point (yellow lines), (2)
 849 K_{leaf} was vulnerable, but not K_{root} (red lines), 3) K_{root} was vulnerable, but not K_{leaf} (light blue
 850 lines), or (4) neither K_{leaf} nor K_{root} was vulnerable (dark blue lines). The inset in (C) shows
 851 cumulative water-use efficiency (WUE; calculated as cumulative CO₂ assimilation (A) over total
 852 transpiration rate (E)) over time. Scenarios including a vulnerable K_{leaf} showed leaves that
 853 showed highest water-use efficiency, cumulative CO₂ assimilation and survived longer under
 854 drought conditions.

855

856 **Figure 8.** Transmitted-electron microscopy of *Arabidopsis thaliana* (Col-0) midrib cross-
 857 sections. In A, the entire xylem portion of the midrib can be seen. Black arrows point to the lack
 858 of secondary lignified wall around xylem conduits. These long primary wall sections can be
 859 observed in more detail in B. The white arrow points to a lignified portion of the secondary

860 xylem wall. We hypothesize that the xylem resistance through these deeply helicoidal xylem
861 conduits is greatly reduced, as unligified primary cells effectively work as one large pit
862 membrane.

863

864 **Supplemental Table and Figure captions**

865 **Supplemental Table S1.** K_LEAF simulation inputs

866 **Supplemental Table S2.** Model inputs and simulation results from MOFLO 2.0.

867 **Supplemental Table S3.** SurEau inputs

868 **Supplemental Figure S1.** External environmental drivers of stomatal conductance measured
869 diurnally in a greenhouse with a porometer.

870 **Supplemental Figure S2.** Leaf water potential is the main driver of observed diurnal variation in
871 stomatal conductance.

872 **Supplemental Figure S3.** Pressure-volume curve for *Arabidopsis thaliana* (Col-0).

873 **Supplemental Figure S4.** Vulnerability curve of the plant (K_{plant} ; brown), leaf (K_{leaf} ; green) and
874 root (K_{root} ; yellow) hydraulic conductance.

875

876 **Table 1.** Modelling framework across scales to determine the underlying mechanisms linking K_{leaf} decline to gas exchange. Symbols:
 877 A_{area} = leaf photosynthetic rate; g_{min} = minimum epidermal conductance; g_{max} = maximum stomatal conductance; g_{s} = stomatal
 878 conductance; K_{leaf} = leaf hydraulic conductance; K_{x} = leaf xylem hydraulic conductance; K_{ox} = leaf outside-xylem hydraulic
 879 conductance; PLC= percent loss of hydraulic conductance; VPD= vapor pressure deficit; Ψ_{leaf} = leaf water potential.

Model	Purpose	Input	Output	Results
K_LEAF (Cochard et al., 2004; Scoffoni et al., 2017)	Model the influence of xylem embolism and potential conduit collapse on K_{x} and K_{leaf}	Leaf size, number of secondary veins and theoretical conductivities from the different veins orders at (1) at full turgor, and after accounting (2) for the decline caused by observed embolism in midrib and/or secondary veins, and (3) for the decline potentially caused by collapsed xylem conduits of tertiary and higher order veins (under a “realistic” collapsed scenario as observed in an oak species (Zhang et al., 2016) which caused 13% PLC, and a more severe scenario—causing 50% PLC)	Leaf xylem hydraulic conductance	Neither embolism nor xylem conduit collapse caused a decline in K_{x} substantial enough to explain the observed decline in K_{leaf} .
MOFLO 2.0 (Buckley et al., 2017)	Model the influence of changes in outside-xylem pathways on K_{ox} and K_{leaf}	Cell shrinkage and % intercellular airspace at -0.5MPa obtained from microCT, stomatal conductance (abaxial and adaxial), vapor pressure deficit and bulk leaf temperature. Simulations were performed under no light or 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, with or without an apoplastic barrier at the bundle sheath, and with or without an 80% decline in cell membrane permeability and/or cell connectivity.	Leaf outside-xylem hydraulic conductance	Reduction of cell membrane permeability in the context of an apoplastic barrier would account for most of the K_{leaf} decline observed at -0.5MPa. Temperature gradients through the leaf due to irradiance had little impact on K_{ox} .
Marginal contribution of K decline (refined from Rodriguez-Dominguez et al., 2016)	Quantify the influence of K_{leaf} decline on g_{s} decline	Parameters from the maximum likelihood function of g_{s} and K_{leaf} vs. Ψ_{leaf} , VPD set at a constant value (1.5 kPa), and a computed range of % g_{s} decline (0 to 100% decline in g_{s} with Ψ_{leaf}).	Contribution of K_{leaf} decline to g_{s} decline with dehydration.	K_{leaf} decline explains most of the changes in g_{s} during mild to moderate dehydration.
SurEau (Martin-StPaul et al., 2017)	Quantify the influence of K_{leaf} decline on gas exchange in whole plant context during drought	Parameters from the maximum likelihood function of K_{leaf} vs. Ψ_{leaf} , parameters from the function of K_{root} vs. water potential, g_{min} , g_{max} , Farqhar’s model inputs, PAR, air temperature, air humidity, time of day, transpiration under well-hydrated conditions, soil volume.	Soil water reserve, water potentials, transpiration rate, g_{s} , A_{area} , PLC.	Decline in K_{leaf} causes leaf water potential to drop, which in turn causes both g_{s} and A_{area} to decline under increasing VPD and decreasing soil water potential.

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883

884 **Table 2.** Mean ± standard error for the physiological and anatomical traits measured for *Arabidopsis thaliana* (col-0). Symbols: *K*_{max}: maximum leaf hydraulic
 885 conductance; *K*_x: leaf xylem hydraulic conductance; %*R*_{ox}: percent resistance outside the leaf xylem; *g*_s: stomatal conductance; *A*_{max}: maximum light
 886 saturated photosynthetic rate; *P*₅₀, *P*₈₈, and *P*₉₅: leaf water potential at 50, 88 ns 95% decline in a given trait.

Trait	Units	Col-0
Hydraulics and gas exchange		
<i>K</i> _{max}	mmol m ⁻² s ⁻¹ MPa ⁻¹	59.9 ± 1.76
<i>K</i> _{leaf0.1-0.2MPa}	mmol m ⁻² s ⁻¹ MPa ⁻¹	33.1 ± 4.55
<i>K</i> _x	mmol m ⁻² s ⁻¹ MPa ⁻¹	138.4 ± 14.5
<i>K</i> _{ox}	mmol m ⁻² s ⁻¹ MPa ⁻¹	106
% <i>R</i> _{ox}	%	54.4
% <i>R</i> _{leaf}	%	85.7
<i>g</i> _s	mmol m ⁻² s ⁻¹	339 ± 24.9
<i>A</i> _{max}	μmol m ⁻² s ⁻¹	14.4 ± 2.72
<i>K</i> _{leaf} <i>P</i> ₅₀	MPa	-0.17
<i>g</i> _s <i>P</i> ₅₀	MPa	-0.38
<i>A</i> _{area} <i>P</i> ₅₀	MPa	-0.37
<i>K</i> _{leaf} <i>P</i> ₈₈	MPa	-0.72
<i>g</i> _s <i>P</i> ₉₅	MPa	-0.71
<i>A</i> _{area} <i>P</i> ₉₅	MPa	-0.71
Drought-tolerance traits		
Turgor loss point (TLP)	MPa	-0.73
Osmotic potential at full turgor (<i>π</i> ₀)	MPa	-0.63
Modulus of elasticity (<i>ε</i>)	MPa	5.70
Relative water content at turgor loss point (<i>RWC</i> _{TLP})	%	84.1
Leaf mass per unit leaf area (LMA)	g m ⁻²	13.6 ± 0.89
Percent loss of area in a dry leaf (<i>PLA</i> _{dry})	%	57.9 ± 3.05
Minimum epidermal conductance (<i>g</i> _{min})	mmol m ⁻² s ⁻¹	18.6 ± 1.33
Leaf anatomical traits		
Distance from vein to lower epidermis (VED)	mm	0.067 ± 0.002
Total vein length per area (VLA)	mm mm ⁻²	3.04 ± 0.08
Minor vein length per area (minor VLA)	mm mm ⁻²	1.79 ± 0.08
Major vein length per area (major VLA)	mm mm ⁻²	1.25 ± 0.05
<i>K</i> _{t, midrib per leaf area}	mmol m ⁻¹ s ⁻¹ MPa ⁻¹	0.27 ± 0.12
<i>K</i> _{t, minor per leaf area}	mmol m ⁻¹ s ⁻¹ MPa ⁻¹	0.003 ± 0.0008

887

888 **Table 3.** Observations of embolized conduits and dimensions from microCT and their simulated impact on leaf xylem hydraulic
 889 conductance using the spatially explicit K_LEAF model. Because we did not have the resolution to determine whether conduit collapse
 890 occurs in tertiary and minor veins, two simulations were performed based on minor vein conduit collapse observed in *Quercus rubra*
 891 (see *Methods*). Note that two leaves were imaged at each water potential (22 leaves total); embolism at the four water potentials below
 892 were only found in one of the two leaves tested at that water potential.

Leaf water potential (MPa)	Number of embolized conduits			Length of embolized conduit (μm)	Diameter of embolized conduit (μm)		Simulated percent loss of xylem hydraulic conductance (%)		
	<i>midrib</i>	2°	3°+	<i>midrib</i>	<i>midrib</i>	2°	<i>Embolism only</i>	<i>Embolism + “realistic” 3°+ vein collapse (13% PLC)</i>	<i>Embolism + “severe” 3°+ vein collapse (50%PLC)</i>
-0.06	0	0	0	-	-	-	0	-	-
-0.08	0	0	0	-	-	-	0	-	-
-0.13	1	1	0	>898	7.3	8.7	7.13	9.99	19.1
-0.16	0	0	0	-	-	-	0	-	-
-0.18	2	2	0	258; >600	6.2; 5.3	5.2; 3.5	3.96	6.70	16
-0.45	0	1	0	-	-	5.2	0.96	3.12	9.1
-0.48	1	0	0	>850	5.9	-	1.16	3.96	13
-0.50	0	0	0	-	-	-	0	-	-
-0.69	0	0	0	-	-	-	0	-	-
-0.79	0	0	0	-	-	-	0	-	-
-0.87	0	0	0	-	-	-	0	2.82	12

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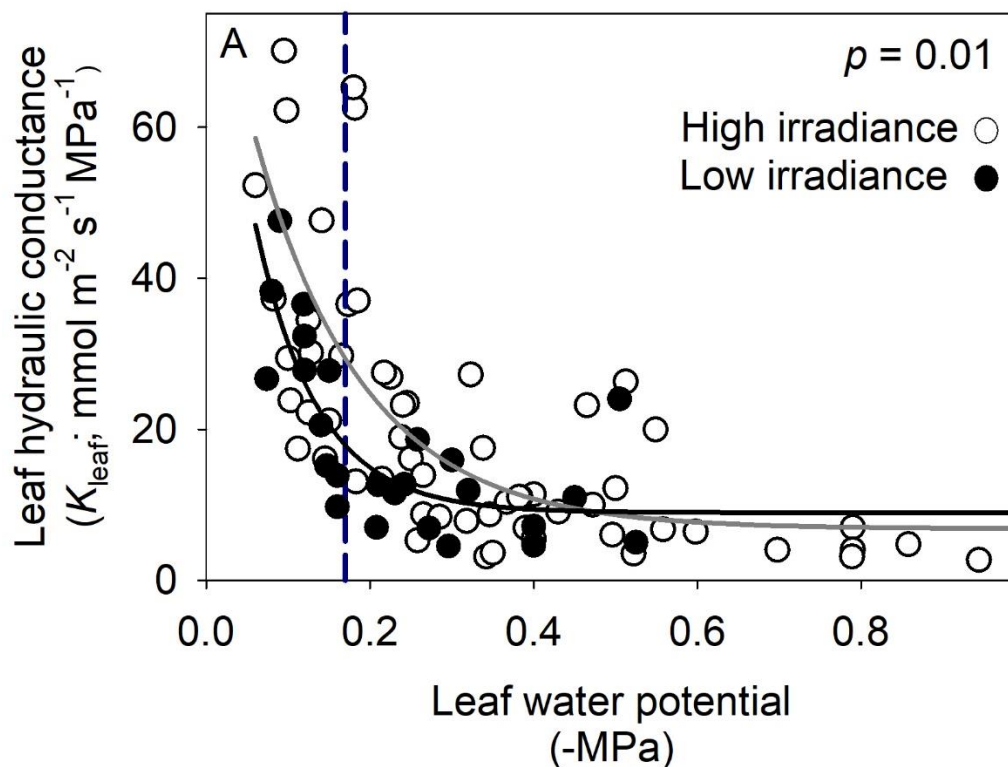


Figure 1. Decline of leaf hydraulic conductance (K_{leaf}) measured under high ($>1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low ($<3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) irradiance. The maximum likelihood function is shown for K_{leaf} vulnerability acclimated under high light ($K_{\text{leaf}} = 6.83 + 81.4 \exp(-7.56 \times |\Psi_{\text{leaf}}|)$), and low light ($K_{\text{leaf}} = 8.98 + 84.2 \exp(-13.2 \times |\Psi_{\text{leaf}}|)$). The dashed line represents the water potential at 50% loss of K_{leaf} (similar in both treatments).

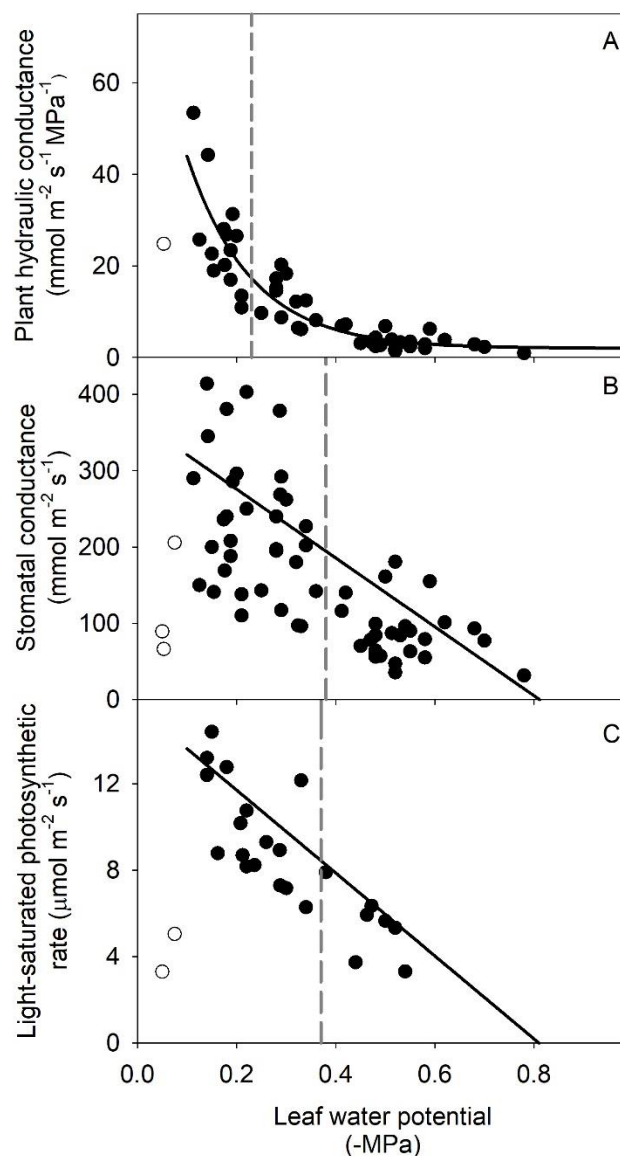


Figure 2. Plant hydraulic and gas exchange response to dehydration in *Arabidopsis*. Decline of the whole plant hydraulic conductance (K_{plant} ; A), stomatal conductance (g_s ; B) and light-saturated photosynthetic rate (A_{area} ; C) with dehydration. Each point represents a different measured leaf. K_{plant} was obtained from the porometer data by dividing transpiration by leaf water potential (assuming soil water potential was at full saturation). The black fitted line in each panel is the maximum likelihood function (exponential for $K_{\text{plant}} = 2.0 + 91.1 \exp(-7.75 \times |\Psi_{\text{leaf}}|)$; linear for $g_s = 339 - 451 \times |\Psi_{\text{leaf}}|$, and $A_{\text{area}} = 14.4 - 19.2 \times |\Psi_{\text{leaf}}|$). The dotted grey line is the leaf water potential (Ψ_{leaf}) at 50% loss of maximum K_{plant} , g_s or A_{area} . Because trait values above -0.1 MPa were especially low (white circles), likely representing stomatal

closure at those high water potentials (see *Methods*), we did not include these points in the line fitting.

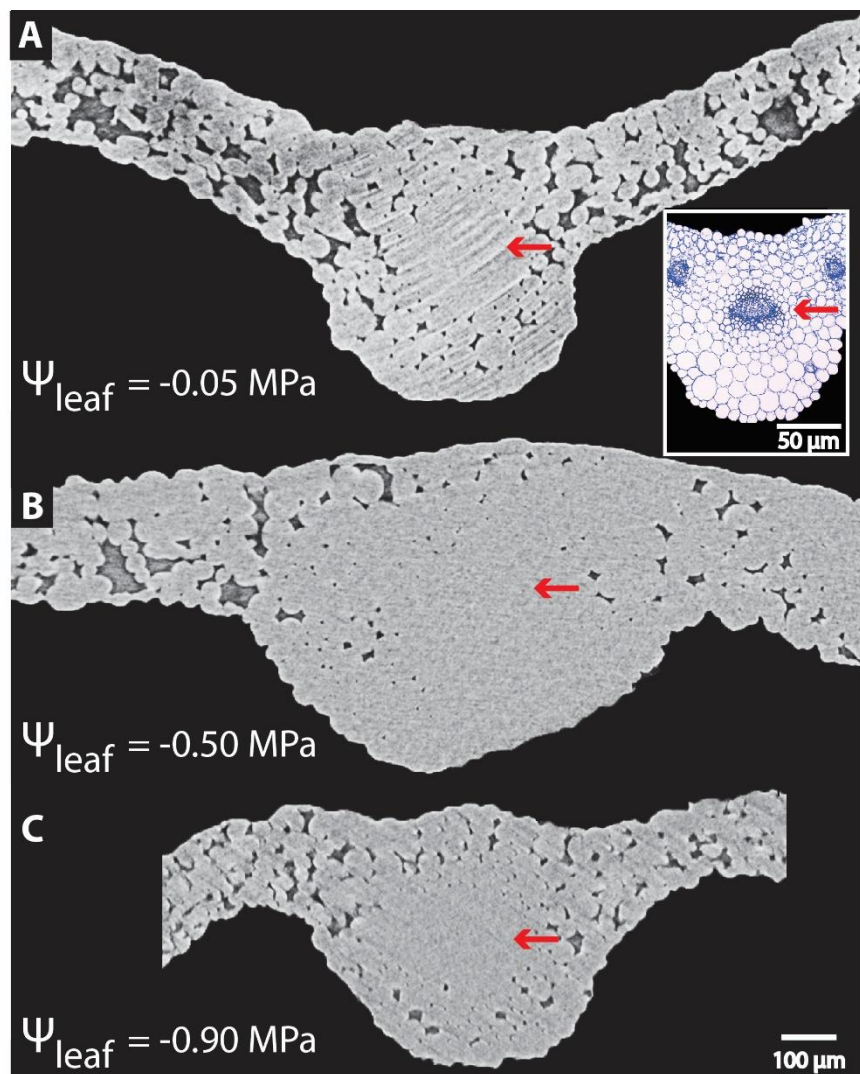


Figure 3. Lack of embolism observed in midrib conduits of *Arabidopsis thaliana* (Col-0) across levels of dehydration as revealed by in vivo images of leaf midribs subjected to progressive dehydration using micro-computed tomography (A-C). Water-filled cells appear in light grey in microCT. If air-filled (i.e., embolized) conduits were present, they would appear as black in the xylem portion of the midrib. There was no embolism, as shown in these images by the red arrows pointing at the entirely light grey midrib xylem. The leaf water potential (Ψ_{leaf}) has been provided for each image. The inset in (A) represents a leaf midrib cross-section imaged under light microscopy, with the red arrow pointing to the xylem tissue (dark blue conduits).

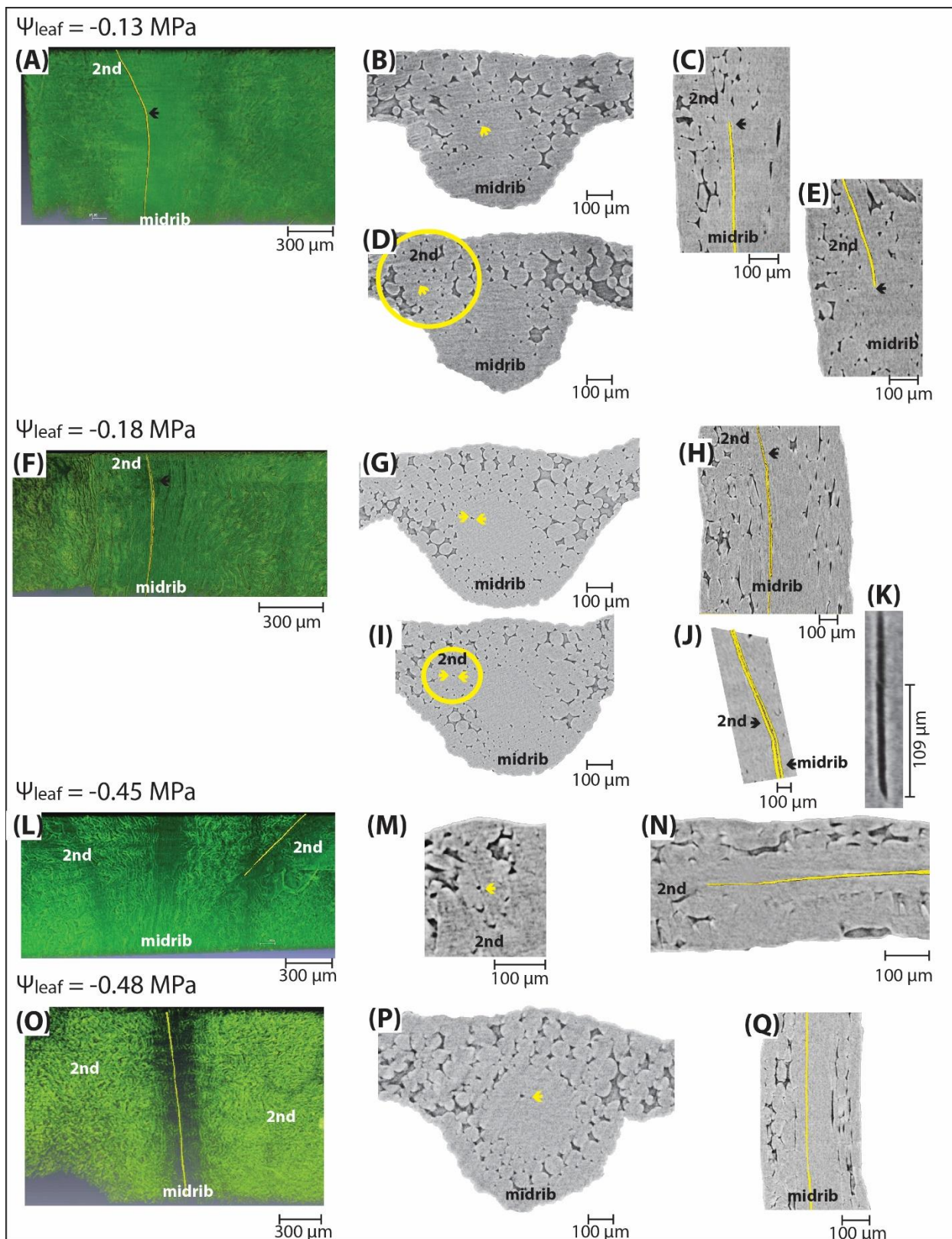


Figure 4. Rare embolisms were observed in a few individual leaves. In two samples, an embolized conduit was observed in the midrib; it continued into a secondary vein (A, F; the

embolized conduits are depicted in yellow). The embolized conduit in the midrib and secondary vein can be seen in cross-sections (B, D, G, I) and longitudinal sections of the microCT scan (C, E, H, J). The arrows point to the embolized conduit (appearing as black in the microCT image). Because of the two dimensionalities of these sections, embolism in the midrib and secondary vein might appear disconnected (C, E). Note that while the embolism was present in only one conduit per cross-sectional image, multiple conduits spanned the length of the midrib and secondary vein (as can be observed in K, where two conduits can be seen connected to one another). Most likely, a first conduit in the midrib embolized, and all the conduits directly connected to that one upstream embolized after. In one sample, an embolized conduit was observed isolated in the secondary vein (L-N), while in another sample, an embolized conduit was observed spanning the midrib length (O-Q).

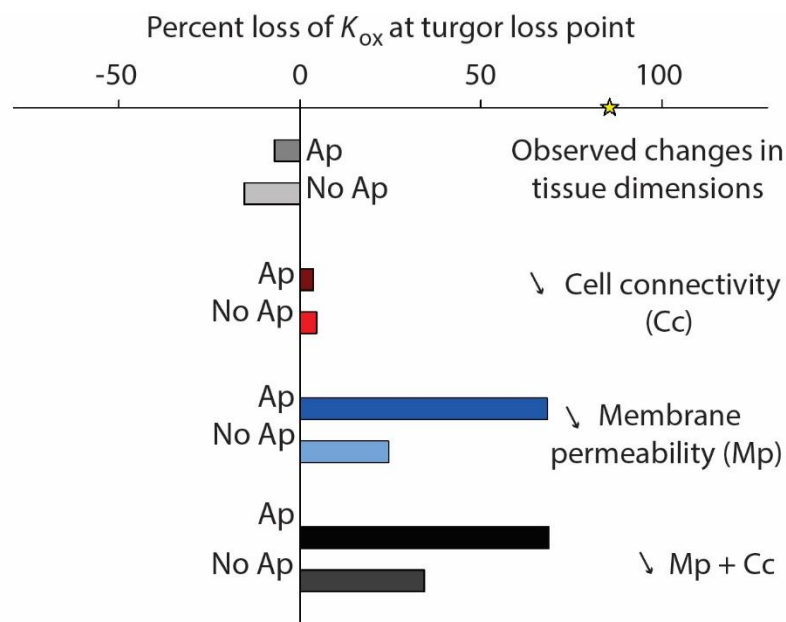


Figure 5. Results from simulations using a spatially explicit model of leaf outside-xylem water to test for potential drivers of the decline in K_{ox} in dehydrating leaves transport (MOFLO 2.0, see Table 1 and *Methods*). The K_{ox} was first computed based on the decline of observed cell size and air space alone (grey bars), which resulted in an increase in K_{ox} (negative percent loss of K_{ox} ; mainly due to shortening of pathways from the veins to stomata). We then modelled K_{ox} decline according to three scenarios (though always including the effect of tissue dimensional changes): an 80% decline at -0.5MPa in (1) cell connectivity (red bars), (2) cell membrane permeability (blue bars), and (3) cell wall thickness (black bars). All simulations were run with (Ap; darker color) or without an apoplastic barrier (No Ap; lighter color) at the bundle sheath cells. The yellow star on the x -axis represents the % observed K_{leaf} decline at -0.5MPa (measured with the evaporative flux method, see *Methods*).

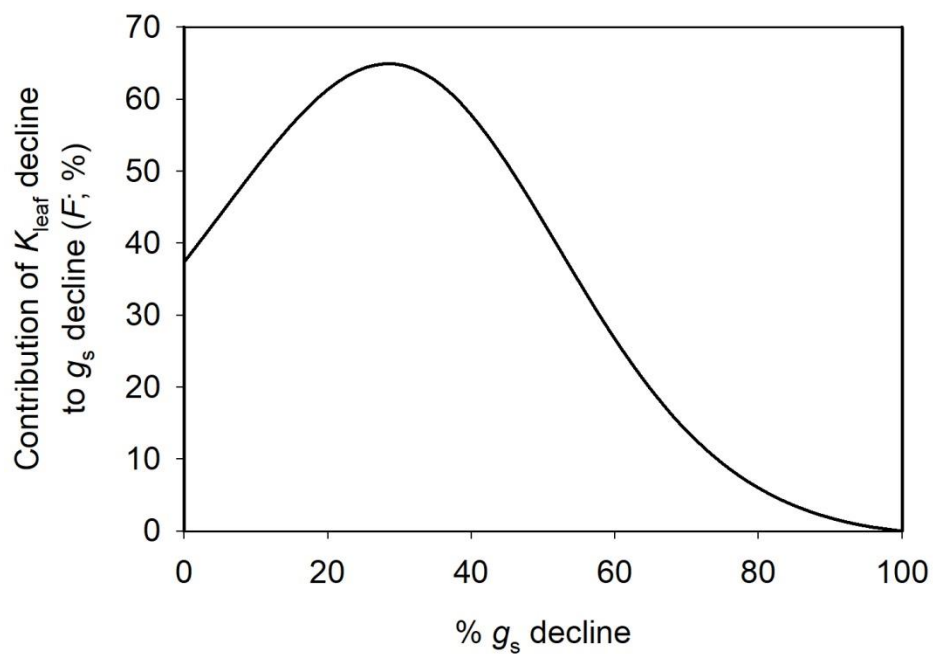


Figure 6. Model simulations mapping the contribution of the decline of leaf hydraulic conductance (K_{leaf}) decline to that of stomatal conductance (g_s) with dehydration (Table 1).

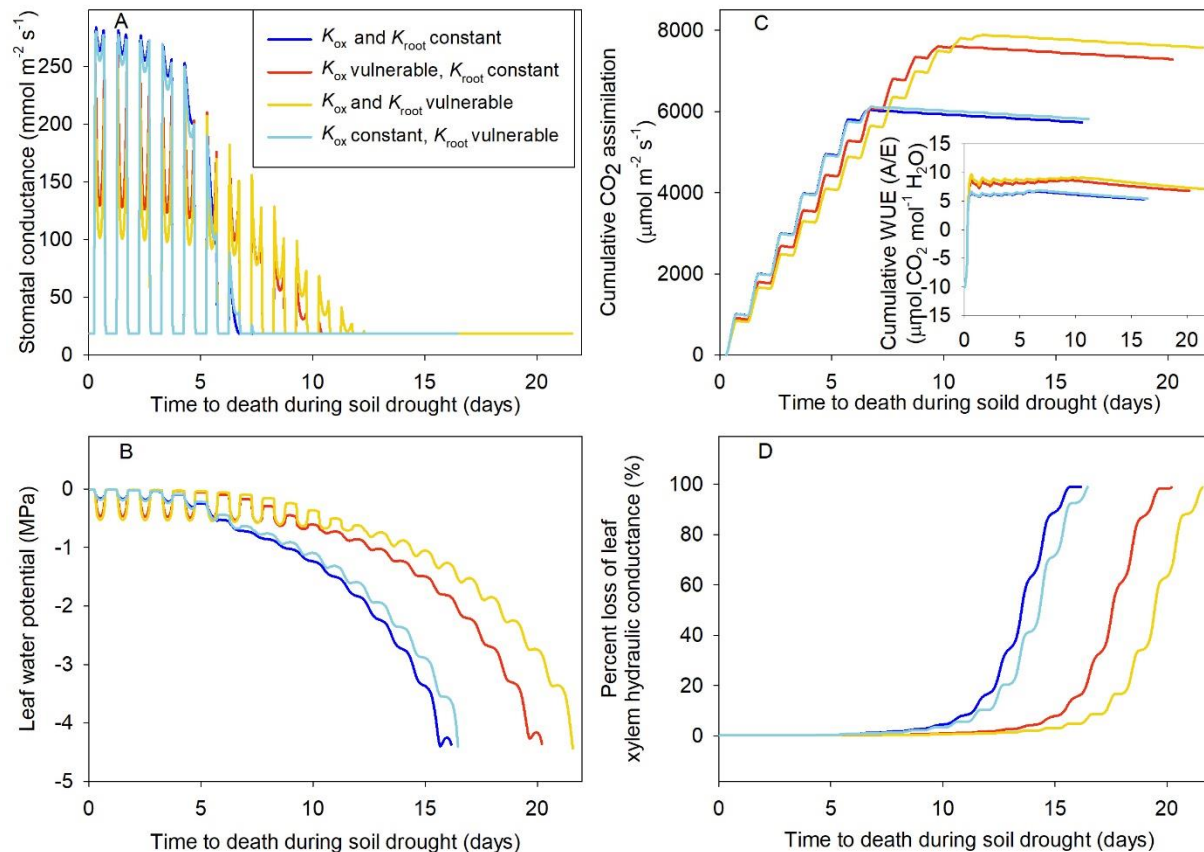


Figure 7. Daily simulated patterns of stomatal conductance (A), leaf water potential (B), cumulative CO₂ assimilation (C) and the percent loss of leaf xylem hydraulic conductance (D) during the progression of a simulated soil drought (SurEau Model, see Table 1 and *Methods*). Four scenarios were modelled: (1) both leaf hydraulic conductance (K_{leaf}) and root hydraulic conductance (K_{root}) were vulnerable to dehydration prior to turgor loss point (yellow lines), (2) K_{leaf} was vulnerable, but not K_{root} (red lines), 3) K_{root} was vulnerable, but not K_{leaf} (light blue lines), or (4) neither K_{leaf} nor K_{root} was vulnerable (dark blue lines). The inset in (C) shows cumulative water-use efficiency (WUE; calculated as cumulative CO₂ assimilation (A) over total transpiration rate (E)) over time. Scenarios including a vulnerable K_{leaf} showed leaves that showed highest water-use efficiency, cumulative CO₂ assimilation and survived longer under drought conditions.

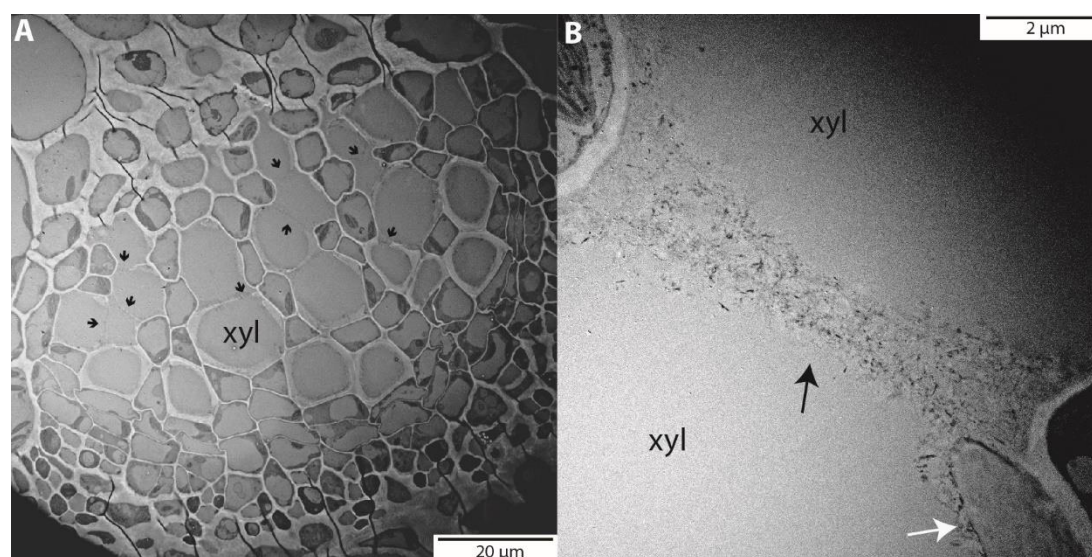


Figure 8. Transmitted-electron microscopy of *Arabidopsis thaliana* (Col-0) midrib cross-sections. In A, the entire xylem portion of the midrib can be seen. Black arrows point to the lack of secondary lignified wall around xylem conduits. These long primary wall sections can be observed in more detail in B. The white arrow points to a lignified portion of the secondary xylem wall. We hypothesize that the xylem resistance through these deeply helicoidal xylem conduits is greatly reduced, as unligified primary cells effectively work as one large pit membrane.

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