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Seasonal and long-term consequences of esca on grapevine stem xylem integrity

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SUMMARY

- Hydraulic failure has been extensively studied during drought-induced plant dieback, but its role in plant-pathogen interactions is under debate. During esca, a grapevine (*Vitis vinifera*) disease, symptomatic leaves are prone to irreversible hydraulic dysfunctions but little is known about the hydraulic integrity of perennial organs over the short- and long-term.
- We investigated the effects of esca on stem hydraulic integrity in naturally infected plants within a single season and across season(s). We coupled *in vivo* X-ray microtomography visualizations with direct (k_s) and indirect (k_{th}) hydraulic conductivity measurements, and tylose and vascular pathogen detection.
- Although no loss of hydraulic conductivity (PLC) was observed in asymptomatic and 40% of symptomatic stems, 60% of symptomatic stems presented xylem occlusions with subsequent PLC, which could reach critical levels (over 50% PLC). A loss of stem k_s was observed simultaneously, or after, the occurrence of leaf symptoms in the presence of tyloses. The impact of esca on xylem integrity was only seasonal and no long-term impact of disease history was recorded.
- Our study demonstrated how and to what extent a vascular disease such as esca, affecting xylem integrity, could amplify plant mortality by hydraulic failure.

Key words: Esca, hydraulic failure, plant dieback, vascular pathogens, *Vitis vinifera* L., tyloses, X-ray microCT, xylem anatomy

47 # INTRODUCTION

48 In agricultural and forest ecosystems, perennial plant dieback causes decreases in plant
49 productivity and longevity (Aleemullah & Walsh, 1996; Eskalen *et al*., 2013; Urbez-Torres *et*
50 *al*., 2013; Alvindia & Gallema, 2017). Plant dieback is a complex process where different biotic
51 and/or abiotic stress factors interact and contribute to leaf and crown wilting and ultimately
52 plant death (Desprez-Lostau *et al*., 2006; Anderegg *et al*., 2013, Cailleret *et al*., 2017;
53 Bettenfeld *et al*., 2020). Drought-mediated plant dieback has been extensively studied, and in
54 this case hydraulic failure has been identified as the primary cause of plant death (Anderegg *et*
55 *al*., 2016). Hydraulic failure results from an interruption of the ascendant water flow by air
56 embolism or xylem occlusion (Zimmermann, 1983; Tyree & Sperry, 1989). Vascular
57 pathogens, which infect the xylem network (Yadeta & Thomma, 2013), are also important
58 drivers of pathogen-mediated plant dieback (Goberville *et al.*, 2016; Pandey *et al*., 2018; Fallon
59 *et al*., 2020).

60 Vascular pathogens induce wood necrosis, leaf symptoms, and crown defoliation (Beckmann
61 & Roberts, 1995; Pearce, 1996). Their biology and toxic metabolite production has been well
62 studied, in particular using controlled phytotoxicity assays (Andolfi *et al.*, 2011; Akpaninyang
63 & Opara, 2017). However, the possible role of hydraulic failure during pathogen-mediated
64 plant dieback has been poorly investigated, and the underlying physiological mechanisms
65 inducing leaf symptoms are not clear yet (Fradin & Thomma, 2006; McDowell *et al*., 2008).
66 Moreover, the long-term impact (over seasons) and relationships between pathogens, leaf
67 symptom presence, and the hydraulic functioning of the plant are still unknown. During
68 vascular pathogenesis, both air (Pérez-Donoso *et al*., 2016) and non-gaseous (Sun *et al*., 2013;
69 Pouzoulet *et al*., 2019) embolism have been observed. For example, air embolism is thought to
70 accelerate pathogen progression during Pierce's disease (Pérez-Donoso *et al*., 2016), and non-
71 gaseous embolism is associated with occlusion of the xylem conduits by the plant that could
72 slow the disease process while interfering with xylem water transport (Sun *et al*., 2013;
73 Pouzoulet *et al*., 2019).

74 Xylem occlusion, usually through the production of tyloses and gels, is one of the first plant
75 defense mechanisms against vascular pathogens (Pearce, 1996). Xylem parenchyma cells
76 secrete gels and expand into the vessel lumen, forming tyloses, physically blocking pathogen
77 progression (Zimmermann, 1979). Xylem anatomy plays an important role, both for vascular
78 pathogen development (Martin *et al*., 2009; Martín *et al*., 2013; Venturas *et al*., 2014;

79 Pouzoulet *et al*., 2017; 2020) and for tylose formation (Bonsen & Kucera, 1990; De Micco *et*
80 *al*., 2016; Pouzoulet *et al*., 2019). If effective, this occlusion mechanism allows the plant to
81 compartmentalize the infected zone and to generate new tissue around it (CODIT model,
82 Pearce, 1996). Because tyloses can potentially interfere with the hydraulic functioning of the
83 plant, they could exacerbate disease symptoms (Talboys, 1972). Tyloses are usually observed
84 in close proximity to pathogens, as shown in artificial inoculation studies (Clerivet *et al,*. 2000;
85 Rioux *et al*., 2018, among others). However, pathogens frequently proliferate in perennial
86 organs without physically reaching the leaves, thus leaf symptoms are often induced at a
87 distance (Beckmann & Roberts, 1995). A recent study shows that tyloses can be present in
88 symptomatic leaves at a distance from the pathogen niches resulting in decreased leaf hydraulic
89 conductivity (Bortolami *et al*., 2019).

90 Over the last decades, grapevine (*Vitis vinifera* L.) mortality and yield loss have been reported
91 in European, American, and South African vineyards due to esca trunk disease (Cloete *et al*.,
92 2015; Guerin-Dubrana *et al*., 2019). Esca affects mostly mature grapevines (more than seven-
93 years-old), and symptoms include trunk necrosis and leaf symptoms, consisting of "tiger-
94 stripe" necrosis and leaf wilting (Lecomte *et al*., 2012; Claverie *et al*., 2020), which are not
95 regularly expressed season-to-season even within individual vines (Guerin-Dubrana *et al*.,
96 2013; Li *et al*., 2017). While the pathogens responsible for esca-induced trunk necrosis have
97 been identified (Morales-Cruz *et al*., 2018; Brown *et al*., 2020), the underlying mechanisms of
98 leaf and fruit symptoms, and plant death are still poorly understood. Bortolami *et al*. (2019)
99 demonstrated that the two vascular pathogens related to esca (*Phaeomoniella chlamydospora*
100 and *Phaeoacremonium minimum*) were never detected in leaves or in one-year old stems, but
101 always in the trunk (independently from leaf symptom presence). They further showed that
102 esca symptomatic leaves presented significant losses in hydraulic conductivity due to the
103 occlusion of the xylem conduits by tyloses. Together, these results suggest a relevant role of
104 hydraulic failure in esca leaf symptom formation, but whether or not there is a corresponding
105 failure in perennial organs, and the exact timing of these phenomena, are still unknown. As
106 stems and branches are the direct connections between the pathogen niche in the trunk and the
107 observed symptoms in the leaves, the study of stem xylem integrity is crucial in the
108 understanding of leaf symptom formation in the current year and across seasons.

109 In this study, we investigated stem xylem integrity in grapevine during esca leaf symptom
110 formation asking the following questions: (i) Can esca lead to hydraulic failure in perennial

111 organs? (ii) Does stem hydraulic failure occur prior to or after leaf symptom expression, and
112 does it depend on xylem anatomy? (iii) Do long-term symptomatic plants present different
113 xylem anatomy and levels of hydraulic failure from long-term asymptomatic plants? To answer
114 these questions, we transplanted 28-years-old grapevines (*Vitis vinifera* L. cv Sauvignon blanc)
115 from the field into pots to transport, manipulate, and study naturally esca-infected vines. We
116 coupled *in vivo* visualizations of stem xylem functionality (using synchrotron-based X-ray
117 microcomputed tomography) with stem specific hydraulic conductivity measurements (k_s),
118 theoretical hydraulic conductivity estimates (k_{th}), optical observations of vessel occlusions, and
119 pathogen detection during symptom appearance, while comparing plants with different
120 symptom history record.

121

122 # MATERIALS AND METHODS

123 **Plant material**

124 *Vitis vinifera* cv. Sauvignon blanc grafted onto 101-14 MGt were uprooted in winter 2017,
125 2018, and 2019 from a vineyard planted in 1992 located at INRAE Bordeaux-Nouvelle
126 Aquitaine (44°47'24.8"N, 0°34'35.1"W) and transferred into pots. Each of these plants has been
127 surveyed each year in the field since 2012 for esca leaf symptom expression following Lecomte
128 *et al*. (2012), and has been classified yearly as leaf-symptomatic or asymptomatic. Plants were
129 then classified by their long-term symptomatology record: plants asymptomatic from 2012 to
130 2018 (pA, previously asymptomatic), and plants that have expressed symptoms at least once
131 between 2012 and 2018 (pS, previously symptomatic). The uprooting method and greenhouse
132 growth conditions are detailed in Bortolami *et al*. (2019).

133 **Esca symptom notation**

134 The evolution of esca leaf symptoms was surveyed twice a week from June to October 2019
135 on every plant (n=84, Fig. 1). As presented in Fig. 1a, esca symptoms were scored at the stem
136 and whole plant scales. Single stems collected for analyses (both hydraulic measurements or
137 microCT observations) could be noted as: asymptomatic (green leaves and apparently healthy),
138 pre-symptomatic (leaves presenting yellowing or small yellow spots between the veins), tiger-
139 stripe (typical pattern of esca leaf symptoms), or apoplectic (leaves passing from green to
140 wilted in a couple of days). Along the experimentation, entire plants could be noted as

141 asymptomatic (control) or symptomatic (when at least 25% of the canopy was presenting tiger-
142 stripe leaf symptoms). At the end of the experiment (week 40, October 2019) each plant was
143 classified as symptomatic or asymptomatic (control). We were then able to group each stem
144 measured into six different groups (Fig. 1a): one group of stems from control plants
145 (asymptomatic from June to October) and five groups of stems from symptomatic plants: two
146 before symptom appearance (asymptomatic and pre-symptomatic stems); and three after
147 symptom appearance (asymptomatic, tiger-stripe, and apoplectic stems). To clearly
148 differentiate asymptomatic stems collected from symptomatic plants and asymptomatic stems
149 collected from asymptomatic plants, we considered plants (and their stems) that didn't show
150 leaf symptoms during the experiment as control plants (or stems). We investigated whether
151 symptom expression (final symptom notation in October 2019, see Fig. 1) differed between
152 plants with contrasted long-term symptom history (previously asymptomatic vs previously
153 symptomatic, Table 1) using a Chi-square test of independence.

154

155 **X-ray microCT observation**

156 Synchrotron-based microCT was used to visualize the content of vessels and their functionality
157 in esca tiger-stripe and control stems. Three symptomatic plants (presenting tiger-stripe
158 symptoms for 8, 7, and 3 weeks), and one asymptomatic-control plant were brought to the
159 PSICHE beamline (King *et al.*, 2016) at SOLEIL synchrotron facility in September 2019.
160 Shoots (ca. 2 m long) were cut under water and transferred into a solution containing 75mM of
161 contrasting agent iohexol. The iohexol solution absorbs X-rays very strongly and appears bright
162 white in X-ray scans above the iodine K-edge at 33.2 keV, and, once it has been taken up by
163 the transpiration stream, the effective functionality of each vessel can be confirmed (Pratt &
164 Jacobsen, 2018; Bortolami *et al.*, 2019). These stems were moved and left outdoor to transpire
165 the solution for at least half a day. The stems were then transferred to the beamline stage and
166 scanned twice in less than 5 minutes using two different energies of a high-flux (3 x 10^{11}
167 photons mm^{-2}) monochromatic X-ray beam: 33.1 keV and 33.3 keV. The projections were
168 recorded with a sCMOS camera equipped with a 250-mm-thick LuAG scintillator (Orca Flash,
169 Hamamatsu, Japan). The complete tomographic scan included 1500 projections, and each
170 projection lasted 50 ms. Tomographic reconstructions were performed using PyHST2 software
171 (Mirone *et al.*, 2014) using the Paganin method (Paganin *et al.*, 2002), resulting in 32-bit

172 volume reconstructions of 2048 x 2048 x 1024 voxels. The final spatial resolution was 2.8769
173 μm^3 $voxel^{-1}$.

174 **Image analysis of microCT scans**

175 The contrast agent iohexol allowed us to distinguish in intact scans the effective functionality
176 of each vessel. In the absence of iohexol, X-ray microCT scans are used to distinguish air-filled
177 vessels (appearing black, corresponding to native PLC) from sap-filled vessels (appearing
178 grey). The addition of iohexol in the xylem sap allows to distinguish the functional vessels
179 (they appear bright white when they transport the sap), from the non-functional ones (i.e.
180 occluded vessels remaining grey, corresponding to occlusion PLC). We could also observe
181 partially occluded vessels (i.e. vessels with simultaneous presence of air and occlusions, or sap
182 and occlusions). This specific case was observed by checking the presence of any occlusion in
183 at least 200 slices in each volume. Partially occluded vessels were considered as occluded,
184 some examples are presented in Fig. S1. The equivalent-circle diameter of air-filled, occluded,
185 and functional (iohexol-filled) vessels was measured on the cross sections from the central slice
186 of the microCT scanned volume using ImageJ software (Schneider et al., 2012). Native PLC,
187 occlusion PLC, and total PLC (i.e. summing occlusion with native PLC) were calculated with
188 the Hagen-Poiseuille equation, as in Bortolami *et al*. 2019, (see Method S1). We investigated
189 whether native PLC, and occlusion PLC differed between control and esca tiger-stripe plants,
190 using two independent generalized mixed linear models where plants were treated as a random
191 effect. Proportional data (ranging from 0 to 1, dividing all PLC values by 100) was analyzed
192 to fit a logit link function and binomial distribution as appropriate.

193

194 **Monitoring stem hydraulic properties over time**

195 Stem xylem integrity was monitored over time by measuring hydraulic properties in control
196 and symptomatic plants along the season and during esca development. Stem specific hydraulic
197 conductivity (k_s) was measured by the gravity method (Sperry et al., 1988), and compared to
198 its theoretical analog (k_{th}) calculated from xylem anatomical observations on the same stem
199 (see below). When there are observed differences in k_s among stems, comparisons with
200 theoretical maximums (k_{th}) can show if lower k_s values result from anatomical differences (i.e.
201 different vessel size distributions) or by hydraulic failure (in the case of similar vessel size and
202 density). If k_s varies in unity with k_{th}, differences in k_s might result from anatomical differences

203 (e.g. smaller k_s are related to smaller vessels), otherwise k_s variations are the consequence of
204 hydraulic failure. Each method to measure k_s, k_{th}, and to observe tyloses is described below.

205 Sampling started on June 19th and finished on September 13th 2019 for a total of 10 sampling
206 dates, 39 stems from 23 control-asymptomatic plants, and 49 stems from 17 symptomatic
207 plants. We randomly sampled control plants and esca symptomatic plants all along the season
208 through the evolution of esca symptoms, obtaining measurements from 14 weeks before until
209 10 weeks after symptom appearance. To explore the contribution of the experimental design to
210 data analysis, we tested the effect of the year of uprooting (2018 and 2019), the position of the
211 analyzed internode, and the week of the measurement (i.e. evolution during the season) on k_s
212 and k_{th} in control plants using separate generalized linear mixed model with normal
213 distributions and the plant treated as a random variable (Table S1). A significant impact of the
214 year of uproot was found for k_s and k_{th} values in control plants (Table S1). This could have
215 resulted from the more favorable conditions (i.e. climatic stability and nutrient availability) for
216 the greenhouse grown vines (note that plants uprooted in 2017 were only esca symptomatic
217 and were not included in this analysis). However, once k_s and k_{th} are plotted together (Fig. S2),
218 all the values lie on the same regression line without generating outlier values (smaller k_s values
219 correspond to smaller k_{th} values independently of the uprooting year).

220 ***Stem specific hydraulic conductivity* (k_s)**

221 k_s measurements were performed on one internode of >1.5m long one-year old stems, following
222 Torres-Ruiz *et al*. (2012) gravity method (see Method S2 for details). A flow of 20 mM KCl
223 solution passed through the sample from a reservoir to a precision electronic balance
224 (AS220.R2, RADWAG, Radom, PL) recording the weight every 5 seconds using the
225 WinWedge v3 5.0 software (TAL Technologies, Philadelphia, PA, USA). Hydraulic
226 conductance, k [kg s^{-1} MPa^{-1}] was obtained by the slope generated by the flow and the
227 corresponding pressure. The linear relationship between flow and pressure obtained were
228 always characterized by R^2>0.97. Stem specific hydraulic conductivity, k_s [kg s^{-1} MPa^{-1} m^{-1}],
229 was calculated as follows:

230 $$k_s = (k \times l) / A$$

231 $$A = ((d_1/2)^2 \times \pi) - ((d_2/2)^2 \times \pi)$$

232 Where: k is the hydraulic conductance, l is the length of the sample, A is the xylem area, d_1 is
233 the external diameter of the debarked stem, and d_2 is the diameter of the central pith.

234 ***Stem theoretical hydraulic conductivity (k_{th}), vessel anatomy, and tylose observation***

235 Just before hydraulic conductivity measurements, the lower internode was stored at 4 °C in
236 80% ethanol for analysis of xylem anatomy. When possible, the same internode of k_s
237 measurements was used for anatomical analysis and k_{th} estimations, otherwise the stored
238 internode was used for the following protocol. 50 µm thick slices were obtained using a GSL-
239 1 microtome (Gärtner *et al.*, 2014). Slices were stained using a 0.5% safranin solution during
240 5 minutes, and then washed three to four times in ethanol (100%). They were quickly soaked
241 in xylene and mounted on microscope slides with Permount Mounting Medium (Electron
242 Microscopy Science, Hatfield, PA, USA). Images were captured with a stereo microscope
243 SMZ1270 (Nikon, France) mounted with a DS-Fi3 camera (Nikon, France). The theoretical
244 conductivity of each vessel (k_{vessel}) [kg m Mpa^{-1} s^{-1}] was calculated using the Hagen-Poiseuille
245 equation:

246 $$k_{vessel} = (\pi \times Ø^4 \times \rho) / (128 \times \eta)$$

247 Where $Ø$ is the equivalent circle diameter [m] (measured with ImageJ software), ρ the density
248 of water [998.2 kg m^{-3} at 20 °C], and η the viscosity of water [1.002 x 10^{-9} MPa s at 20 °C]. k_{th}
249 of the stem [kg s^{-1} m^{-1} Mpa^{-1}] was then calculated by summing every k_{vessel} in the xylem area
250 (A) [m²]:

251 $$k_{th} = \Sigma\, k_{vessel} / A$$

252 In the entire cross section of each sample, the physical presence (or absence) of tyloses in vessel
253 lumina was visually assessed.

254 Regarding the statistical analysis, stems were grouped in six different categories following their
255 esca symptomatology (as presented in Fig. 1a). We investigated whether k_s, k_{th}, and total vessel
256 density differed among these different categories, and how k_s, k_{th}, and total vessel density
257 differed between stems with and without tyloses (independently from leaf symptom presence),
258 using independent mixed linear general models. The symptom / tylose category and the year
259 of uprooting (since it had a significant impact on k_s and k_{th} in control plants, Table S1) were
260 entered as fixed effects, with the plant treated as a random effect since different stems were

261 sometimes analyzed from the same plant (88 analyzed stems on 40 different plants). Total
262 density and densities for each vessel diameter class were log-transformed prior to analysis to
263 fit normality requirements. For the classes with no vessels (e.g. samples without vessel
264 diameters above 160 µm), a minimal density of 0.0001 was assigned prior to log
265 transformation. We investigated whether the frequency of symptomatic stems presenting
266 tyloses changed with the symptom age (i.e. weeks between first symptom detection and k_s
267 measurements on the same plant) with a Chi-square test. The relationships between stem k_s and
268 k_{th} were tested using linear regression models. Finally, we investigated whether k_s and k_{th} in
269 control stems differed between plants with different symptom history records using
270 independent mixed linear general models with the plant treated as a random effect.

271

272 **Fungal detection**

273 Detection and quantification of *Phaeomoniella chlamydospora* and *Phaeoacremonium*
274 *minimum* were performed using qPCR in a subsample of stems and trunks from the same
275 symptomatic and control plants used for hydraulic and anatomical measurements. All along the
276 season, basal internodes, from the same stems sampled for k_s and k_{th} measurements, were
277 directly placed in liquid nitrogen and stored at -80 °C. At the end of the experiment, a subset
278 of plants was cut at the base for trunk sampling. A 2 cm high section was cut with a sterilized
279 hand saw. The bark was removed and the different tissues of each section (necrotic and
280 apparently healthy wood) were separately collected using ethyl alcohol-sterilized shears in a
281 sterile environment, and immediately placed in liquid nitrogen. All samples were ground in
282 liquid nitrogen using a tissue lyser (Tissuelyser II, Qiagen, Germantown, MD, USA). DNA
283 extraction and qPCR analysis were conducted as described in Pouzoulet *et al*. (2013) using the
284 primer sets PchQF/R and PalQF/R. Detection and quantification of *P. chlamydospora* and *P.*
285 *minimum* DNA by qPCR (SYBR Green assays) was conducted as described by Pouzoulet *et*
286 *al*. (2017). After qPCR analysis, the results from each trunk sample (i.e. necrotic or apparently
287 healthy wood) were averaged together in order to obtain one quantification per plant. Pathogen
288 DNA quantity (average value of three technical replicates, fg/µl) was normalized by the amount
289 of total DNA (ng/µl), detected using a Qubit fluorometer. We investigated whether the amount
290 of fungal DNA (both for *P. chlamydospora* and for *P. minimum*) in trunks differed between
291 symptomatic and control plants, and between control plants with different symptom history

292 records, using generalized linear mixed model with a poisson distribution and a log likelihood
293 function.

294

295 **Statistical analysis**

296 All data management and statistical tests were done in SAS software (SAS 9.4; SAS Institute).
297 We used PROC GLIMMIX for generalized linear mixed models, PROC GLM for generalized
298 linear models, PROC REG for regression analyses and PROC FREQ for frequency analyses
299 (Chi-square test of independence). The normality of the response variables was tested using a
300 Kolmogorov-Smirnov test (PROC UNIVARIATE) prior to analyses. Data were log-
301 transformed (total density) or appropriate distributions (binomial, poisson) were fitted when
302 appropriate.

303

304 # RESULTS

305 **Esca leaf symptom expression within and across seasons**

306 Esca leaf symptoms were recorded in 20 out of the 58 plants followed in this study (35%, Fig.
307 1, Table 1). The number of symptomatic plants increased gradually with time, from the first
308 symptom appearance in early June to the last in late September (Fig. 1). There was no effect of
309 the plant history (previously asymptomatic pA, or previously symptomatic pS) on 2019
310 symptom expression (n=58, X^2=0.27, P=0.60). On 20 pA plants, six (30%) expressed leaf
311 symptoms in 2019 (Table 1). On 38 pS plants, fourteen (37%) showed symptoms in 2019
312 (Table 1). However, pS plants expressed symptoms from June to the end of September, while
313 pA plants showed leaf symptoms only in September.

314

315 ***In vivo* observations of esca symptomatic stems**

316 Xylem vessels of control and tiger-stripe stems were observed using three dimensional X-ray
317 microCT scans in iohexol-fed samples (Fig. 2, 3, Table S2). As shown in Fig. 2, functional and
318 non-functional vessels can be discriminated through the use of iohexol (functional vessels
319 appear bright white, non functional vessels appear either black if air-filled or grey if occluded).

320 We observed almost totally functional stems in all asymptomatic stems (<20% total PLC, Fig.
321 2a-c), and 40% of tiger-stripe stems (e.g. Fig. 2d-g). Higher levels of PLC (>20% total PLC,
322 Fig h-m) were observed in the remaining tiger-stripe stems, with 40% of tiger-stripe stems
323 exhibiting over 50% total PLC (Fig. 2j-m). When the two components of PLC were
324 disentangled, we observed that the level of native PLC remained low both in control (6.5 ±
325 2.6%) and in tiger-stripe (12.2 ± 2.9%) stems (Fig. 3a). Occlusion PLC values were virtually
326 zero in control stems (0.7 ± 0.02%) while in tiger-stripe stems the mean occlusion PLC values
327 was 27.5 ± 8.2% (Fig. 3b). Nevertheless, the variability of occlusion PLC across tiger-stripe
328 stems was very high, the values ranging from 0.3% to 72.9% (Fig. 2d-m, and 3b), and occlusion
329 PLC was not correlated to symptom age (n=10, $F_{2,7}$=0.19, P=0.83). Consequently, no statistical
330 differences in native or occlusion PLC were found between control and tiger-stripe stems (Fig.
331 3). When higher occlusion PLC was measured (Fig. 2h-m), occluded vessels could be
332 organized either on one side of the stem (Fig 2j-l) or randomly distributed across the section
333 (Fig 2h, 2i, 2m). In 90% of symptomatic stems, we observed that the most external vessels
334 were functional. Occlusions were present equally in all vessel diameter classes (Fig. S3).

335

336 **Tylose development, stem specific (k_s) and theoretical (k_{th}) hydraulic conductivity during**
337 **esca leaf symptom formation**

338 Tyloses were identified in the xylem vessels of certain tiger-stripe stems and throughout the
339 temporal development of esca leaf symptoms, from the appearance of symptoms to 11 weeks
340 after. All apoplectic stems and 62.5% (15 of 24 analyzed stems) of esca tiger-stripe stems
341 presented tyloses, while all other stems (control, asymptomatic or pre-symptomatic) did not
342 contain these occlusions, even until one week before symptom development. In esca tiger-
343 stripe stems, tyloses were not related to specific plants, or to symptom age (i.e. on the same
344 plant at the same moment, different symptomatic stems could present tyloses, or not, n=24,
345 X^2=7.47, P=0.38).

346 Overall, no significant impact of esca symptoms was observed on k_s (Fig. 4a), even if tiger-
347 stripe stems were divided between those with and without tyloses. Control stems presented a
348 mean (± SE) k_s of 24.97 ± 1.72 kg s^{-1} MPa^{-1} m^{-1}; all the stems without tyloses measured on
349 symptomatic plants showed the same range of values as control stems (Fig 4a, Table 2): 26.04
350 ± 4.71 for asymptomatic before symptoms appearance, 30.32 ± 4.26 for pre-symptomatic

351 stems, 19.80 ± 5.18 for asymptomatic stems after symptom appearance on the plant, and 21.29
352 ± 5.40 for tiger-stripe stems without tyloses. Stems with tyloses (tiger-stripe and apoplectic
353 stems) presented the lowest average k_s values (11.27 ± 2.86 and 2.47 ± 1.45 kg s^{-1} MPa^{-1} m^{-1}
354 for tiger-stripe and apoplectic, respectively). Regarding k_{th}, no significant impact of esca
355 symptoms was found (Fig. 4c, Table 2), all the values were in the same range, with average
356 values ranging from 70.44 (for tiger-stripe stems with tyloses) to 87.88 (for pre-symptomatic
357 stems) kg s^{-1} MPa^{-1} m^{-1}.

358 In order to further investigate the impact of esca on stem hydraulics, we explored the
359 relationship between individual stem k_s and k_{th} in each symptom category (Fig. 4b, S4, Table
360 2). Significant relationships were found between k_s and k_{th} in all groups except in asymptomatic
361 stems after symptom appearance and symptomatic stems with the physical presence of tyloses
362 (Fig. S4, Table 2). The slopes of regression curves between k_s and k_{th} did not vary among
363 groups in the absence of tyloses (slope values ranged between 0.3 and 0.4, Table 2) while it
364 was close to 0 in the presence of tyloses (0.17 for tiger-stripe and 0.04 for apoplectic stems).
365 When k_s and k_{th} are compared in the presence or absence of tyloses, we observed that k_s was
366 significantly lower when tyloses were present (9.81 ± 2.51 kg s^{-1} MPa^{-1} m^{-1} in the presence of
367 tyloses vs 25.06 ± 1.46 kg s^{-1} MPa^{-1} m^{-1} in the absence of tyloses, Table 2, n=88, $F_{1,49}$=7.11,
368 P=0.01) while k_{th} did not significantly differ. Stems without tyloses presented a strong
369 correlation between k_s and k_{th}, while in the presence of tyloses this relationship was not
370 significant (Table 2, Fig. 4b).

371 Total vessel density did not significantly differ between stem symptomatology (comparing all
372 the seven categories presented in Table 2), even when vessel density was partitioned by vessel
373 diameter classes (Fig. 4d).

374 Finally, we tested the impact of disease history (comparing pA and pS plants) on the hydraulic
375 conductivity and xylem anatomy in control plants. There were no differences between long-
376 term symptomatic (pS) and long-term asymptomatic (pA) plants in stem k_s, stem k_{th}, or total
377 vessel density (Table 3).

378

379 **Fungal detection**

380 The two vascular pathogens associated with esca (*Phaeomoniella chlamydospora* and
381 *Phaeoacremonium minimum*) were never detected in one-year old stems while they were
382 systematically detected in the trunk of both control and symptomatic plants (Table 4). In trunks,
383 a significantly higher quantity of fungal DNA was detected in tiger-stripe symptomatic plants
384 than in controls (Table 4). We found 2.14- and 1.64-fold more of *P. chlamydospora* and *P.*
385 *minimum* DNA in symptomatic trunks relative to controls. In control plants, different symptom
386 history record impacted the quantity of fungal DNA detected by qPCR, for *Phaeomoniella*
387 *chlamydospora*, and for *Phaeoacremonium minimum*. We found 1.65- and 2.84-fold more *P.*
388 *chlamydospora* and *P. minimum* DNA in previously symptomatic trunks relative to previously
389 asymptomatic trunks (Table 3).

390

391 # DISCUSSION

392 Our results regarding the xylem integrity during esca show that the presence of plant-derived
393 tyloses induced hydraulic failure in 60% of symptomatic stems. Tyloses were produced
394 simultaneously with, or after, the occurrence of leaf symptoms, and resulted in more than 50%
395 PLC in 40% of symptomatic stems, unrelated to symptom age. We demonstrated that the
396 presence of leaf symptoms during previous seasons had no impact on the likelihood of
397 symptom appearance in the current year, or on stem hydraulic conductivity and xylem anatomy.
398 Vascular fungi were never detected in the same organs as the tyloses (one-year old stems), and
399 although they were present in trunks of both tiger-stripe and control plants, tiger-stripe plants
400 showed higher quantities of fungal DNA. Among control plants that did not express symptoms
401 in the year of the study, we found higher quantities of fungal DNA in those plants with a long-
402 term history of symptom formation. As xylem occlusions were not present in the totality of
403 tiger-stripe stems, they are unlikely to be the direct structural cause of esca leaf symptom.
404 However, they could increase the risk of plant mortality, as they impair water transport in a
405 majority of symptomatic stems.

406

407 ## *In vivo* xylem integrity observations and hydraulic vulnerability segmentation

408 Using direct X-ray microCT imaging in esca symptomatic stems, we found that hydraulic
409 conductivity loss was almost entirely associated with the presence of tyloses. Different studies

410 have investigated the link between vascular pathogen development and hydraulic conductivity
411 in stems (Collins *et al*., 2009; Lachenbruch & Zhao, 2019; Mensah *et al*., 2020). During biotic
412 stresses, air embolisms have been shown to decrease hydraulic conductivity during bacterial
413 leaf scorch disease (McElrone *et al*., 2003; 2008), Pierce's disease (Pérez-Donoso *et al*., 2016),
414 and Pine wilt disease (Yazaki *et al*., 2018). In the case of fungal wilt diseases, the hydraulic
415 conductivity loss was associated with non-gaseous embolism (i.e. tyloses) at the point of
416 pathogen inoculation (Guerard *et al*., 2000; Sallé *et al*., 2008; Beier *et al*., 2017; Mensah *et al*.,
417 2020), or with canker presence in naturally infected stems (Lachenbruch & Zhao, 2019).

418 Using iohexol we were able to visually observe the exact spatial organization of functional
419 vessels. Interestingly, in some symptomatic samples we found functional vessels surrounding
420 the non-functional xylem (Fig. 2j-l), suggesting that the plant was able to preserve the more
421 external vessels from occlusions or to form new functional vessels after the loss of
422 conductivity. Moreover, the sectoriality of the occlusions observed in Fig. 2j-l was reminiscent
423 of the sectoriality observed in the distributions of trunk necrosis, especially on the brown stripe
424 necrosis appearing along the vasculature (Lecomte *et al*., 2012).

425 Comparing these results with our precedent study using the same technique in leaves, we
426 showed that esca symptomatic leaves presented higher levels of occlusion PLC (60.7 ± 6.80%
427 in midribs, and 54.02 ± 8.72% in petioles, data from Bortolami *et al*., 2019) compared to stems
428 (27.53 ± 8.24 %, occlusion PLC), suggesting hydraulic vulnerability segmentation (although
429 PLC in leaves and stems were measured in different plants and years). The hydraulic
430 segmentation theory relies on the fact that annual organs (i.e. leaves) are more vulnerable than
431 perennial organs (i.e. stems) to drought induced air embolism (Tyree & Ewers, 1991).
432 Grapevine is well known for exhibiting strong hydraulic vulnerability segmentation (Charrier
433 *et al*., 2016; Hochberg *et al*., 2016; 2017). This is thought to be adaptive, where the higher
434 vulnerability in leaves and petioles favors embolism formation and leaf shedding prior to
435 embolism formation in stems, thus protecting the perennial organs. Our observations during
436 esca pathogenesis demonstrate that, analogous to the hydraulic vulnerability segmentation
437 theory, leaves appear more vulnerable to the formation of non-gaseous embolism as well,
438 which could mitigate the risk of hydraulic failure in perennial organs. From another
439 perspective, the difference may not be a direct effect of the specific organ's vulnerability to
440 non-gaseous embolism, but a consequence of a difference in the accumulation of putative
441 toxins and/or elicitors. Indeed, we confirmed here that esca leaf symptoms occur at a distance

442 from the pathogen niche because vascular pathogens were never detected in one-year old stems,
443 suggesting that the plant may transport a signal (i.e. toxins or elicitors) from the infected trunk
444 up to the leaves. If the signal accumulates in leaves in a higher amount than it does in the stems,
445 and stimulates occlusion formation, stems would then be secondarily affected.

446

447 **Hydraulic conductivity, tyloses, and vessel anatomy**

448 Tyloses could have different impacts, both positive and negative, during wilt disease
449 pathogenesis: (i) tyloses contribute to pathogen resistance as they aim to seal off vessel lumens
450 and impede pathogens spread throughout the host (CODIT model, Pearce, 1996). This is the
451 case regarding the susceptibility of different species or varieties to specific pathogens (Jacobi
452 & MacDonald, 1980; Ouellette *et al*., 1999; Clerivet *et al.,* 2000; Et-Touil *et al*., 2005; Venturas
453 *et al*., 2014; Park & Juzwik 2014; Rioux *et al*., 2018), in particular to *Phaeomoniella*
454 *chlamydospora*, one of the pathogen associated with esca (Pouzoulet *et al*., 2017; 2020). (ii) In
455 other studies, it has been shown that tyloses can exacerbate symptoms (Talboys, 1972): they
456 cause a reduction in stem hydraulic conductivity, sometimes associated with a reduction in
457 stomatal conductance in leaves and, in the most severe cases, wilting (Parke *et al*., 2007; Beier
458 *et al*., 2017; Lachenbruch & Zhao, 2019, Mensah *et al*., 2020 during fungi development; Sun
459 *et al*., 2013; Deyett *et al*., 2019 during Pierce's disease). Our results suggest that during esca
460 (i) the development of tyloses in stems cannot be interpreted as a systematic trait of pathogen
461 resistance because visual symptoms were observed despite the presence of tyloses and they
462 were produced at a distance from pathogens. (ii) When present, tyloses might lead to symptom
463 exacerbation. Esca has also been suggested to lead to a general reduction in xylem water
464 transport and stomatal conductance (Ouadi *et al*., 2019), and tyloses could be a major
465 contributor to these phenomena as during winter senescence (Salleo *et al*., 2002; Sun *et al*.,
466 2008). However, when stems have no tyloses, esca leaf symptom formation seems to arise from
467 within the leaf itself, and does not result from upstream hydraulic failure.

468 Xylem is the battleground between vascular pathogens and the plant's defense response
469 (Yadeta & Thomma, 2013). Even if xylem vessel anatomy is less investigated, it could have a
470 crucial role in plant resistance and response to vascular pathogens. For example, during Dutch
471 elm wilt disease (due to *Ophiostoma spp.*) the most sensitive species and varieties present wider
472 xylem vessels (Elgersma, 1970; Mcnabb *et al*., 1970; Solla & Gil 2002; Pita *et al*., 2018).

473 Smaller vessels could occlude faster, sustaining a more efficient pathogen restriction (Venturas
474 *et al*., 2014). Our results on xylem vessel anatomy suggest that stems with tyloses tend to
475 present higher densities of small vessels, even if we did not observe any differences in total k_{th}
476 values. Moreover, microCT scans showed that occlusions appear randomly in every vessel size
477 class (Fig. S3). In contrast, artificial inoculations showed that xylem vessel diameter had a
478 strong impact on esca-related vascular pathogen development (Pouzoulet *et al*., 2017; 2020),
479 and in the kinetic of vessel occlusion in grapevine stems (Pouzoulet *et al*., 2019). The
480 relationships between esca leaf symptoms, xylem anatomy, and tylose presence should be
481 studied in detail in trunks, where vascular pathogens are present, and among different grapevine
482 varieties and rootstocks as they are known to show different susceptibility to symptom
483 expression.

484

485 **Long-term consequences of esca on leaf symptom expression and stem hydraulic integrity**

486 In field surveys, esca leaf symptoms are often randomly distributed spatially throughout
487 vineyards and are not consistent from season to season in individual vines (Mugnai *et al*., 1999;
488 Surico *et al*., 2000; Marchi *et al*., 2006; Guerin-Dubrana *et al*., 2013; Li *et al*., 2017). However,
489 esca-related vine death is strongly related to leaf symptoms as death is usually observed
490 following a year with symptom expression (Guerin-Dubrana *et al*., 2013). In agreement with
491 these field studies, we observed similar percentages of symptomatic plants between those that
492 had already expressed esca symptoms in the past (from one to seven consecutive years, pS
493 plants), and those that had never expressed symptoms over the past seven years (pA plants).
494 However, we also found that pS plants expressed symptoms earlier in the season than pA
495 plants, suggesting that symptoms might require more time to develop in pA plants. We did not
496 find any significant differences in k_s and k_{th} values between plants with contrasted long-term
497 symptom history. This result suggests that esca leaf symptoms may have xylem anatomical
498 consequences within the year of expression by the production of tyloses, but not across seasons.
499 Moreover, we showed that DNA pathogen amount (*Phaeoacremonium minimum* and
500 *Phaeomoniella chlamydospora*) depends on the symptom expression in the season of sampling,
501 and on the long-term symptom history. Altogether, these results suggest that a higher amount
502 of vascular fungi in the trunk represents a higher risk in reproducing leaf symptoms, and
503 consequently, a higher risk of plant death.

504

505 **Hydraulic failure and esca leaf symptom pathogenesis**

506 Our results showed that, even if esca-related stem occlusion was extremely variable, 40% of
507 the microCT analyzed stems presented a total PLC greater than 50%. Under drought conditions
508 alone, studies suggest that grapevines are not able to recover in the current season from PLC
509 greater than 50% in stems (Charrier *et al*., 2018). Thus, to what extent these levels of esca-
510 induced hydraulic failure compromise future vine performance, and/or increase the likelihood
511 of developing esca leaf symptoms in the future remains an open question.

512 We showed that, similarly to visual leaf symptoms, tyloses in stems were generated at a
513 distance from the pathogen niche in the trunk. Comparing our results with Bortolami *et al*.
514 (2019), we show that hydraulic failure affected leaves, on average, two times more than stems.
515 We could hypothesize that, following pathogen activities in the trunk, a signal passing through
516 the xylem network and stimulating tyloses, first accumulates in leaves and then affects the
517 stems. However, the exact signal and action remain unknown, as we showed that the presence
518 of tyloses depended upon given symptomatic stems rather than symptomatic plants (i.e. two
519 stems in the same plant, with same tiger-stripe symptoms, sampled at the same moment, could
520 or could not present tyloses).

521 We showed that there were no differences in symptom expression, nor in the stem hydraulic
522 properties, regarding the long-term symptom history. We can conclude that the processes that
523 generate tiger-stripe symptoms are largely restricted to the current year of the symptom
524 expression. However, in plants expressing symptoms for the first time according to our disease
525 record, these processes could require more time, as they showed symptoms only late in the
526 season. The presence of occlusion, leading to hydraulic failure in stems, could exacerbate leaf
527 symptom expression in the following seasons, possibly contributing to death. We could
528 speculate that a stem expressing extensive hydraulic failure could be more prone to express
529 symptoms in the following year or, in the worst cases, to die. If the level of hydraulic failure
530 could affect the stem mortality in the following year, the choice of stems with a complete
531 absence of failure during the winter pruning could reduce the impact of esca in vineyards. In
532 addition, the presence of occlusions could also amplify plant susceptibility to drought-induced
533 hydraulic failure, enhancing the risk of plant mortality in the field as suggested by McDowell
534 et al. (2008). It could be speculated that a decrease in soil water potential or a high evaporative

demand, concomitant to esca-induced hydraulic failure, could embolize the remaining functional xylem vessels stopping the water flow and desiccating plant tissues (this could be the case in apoplectic plants for example). In perspective, future studies should investigate the link between pathogen activities and occlusion development, especially in trunks, and the subsequent hydraulic failure consequences on whole plant physiology.

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Author contributions

C.E.L.D., G.A.G., G.B., and S.D. designed the experiments;
E.F., G.A.G., S.D., E.B., R.B., H.C., A.K., L.J.L., J.M.T.-R., S.T. participated in synchrotron campaigns;
G.B., C.E.L.D., E.F., and N.F. conducted the esca symptom notations;
G.B., M.M.-M.,and N.F. conducted the histological observations;
E.F. conducted the hydraulic conductivity measurements and participated to data analyses;
N.F., and J.P., conducted the pathogen detection;
G.B. analyzed the microCT, optical images, and analyzed the data;
P.L. provided data on disease history of the plants;
G.B., C.E.L.D., and G.A.G. wrote the article;
all authors edited and agreed on the last version of the article.

Data Availability Statement

We agree to archive the data associated with this manuscript should the manuscript be accepted.

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836 **SUPPORTING INFORMATION**

837 The following Supporting Information is available for this article:

838 **Method S1:** Image analysis of microCT scans.

839 **Method S2.** Specific stem hydraulic conductivity (k_s) measurements.

840 **Fig. S1 (a-d)**. Two-dimensional reconstruction of longitudinal cross sections from X-ray
841 microCT volumes of grapevine stems.

842 **Fig. S2**. Relationship between k_s and k_{th} in control plants.

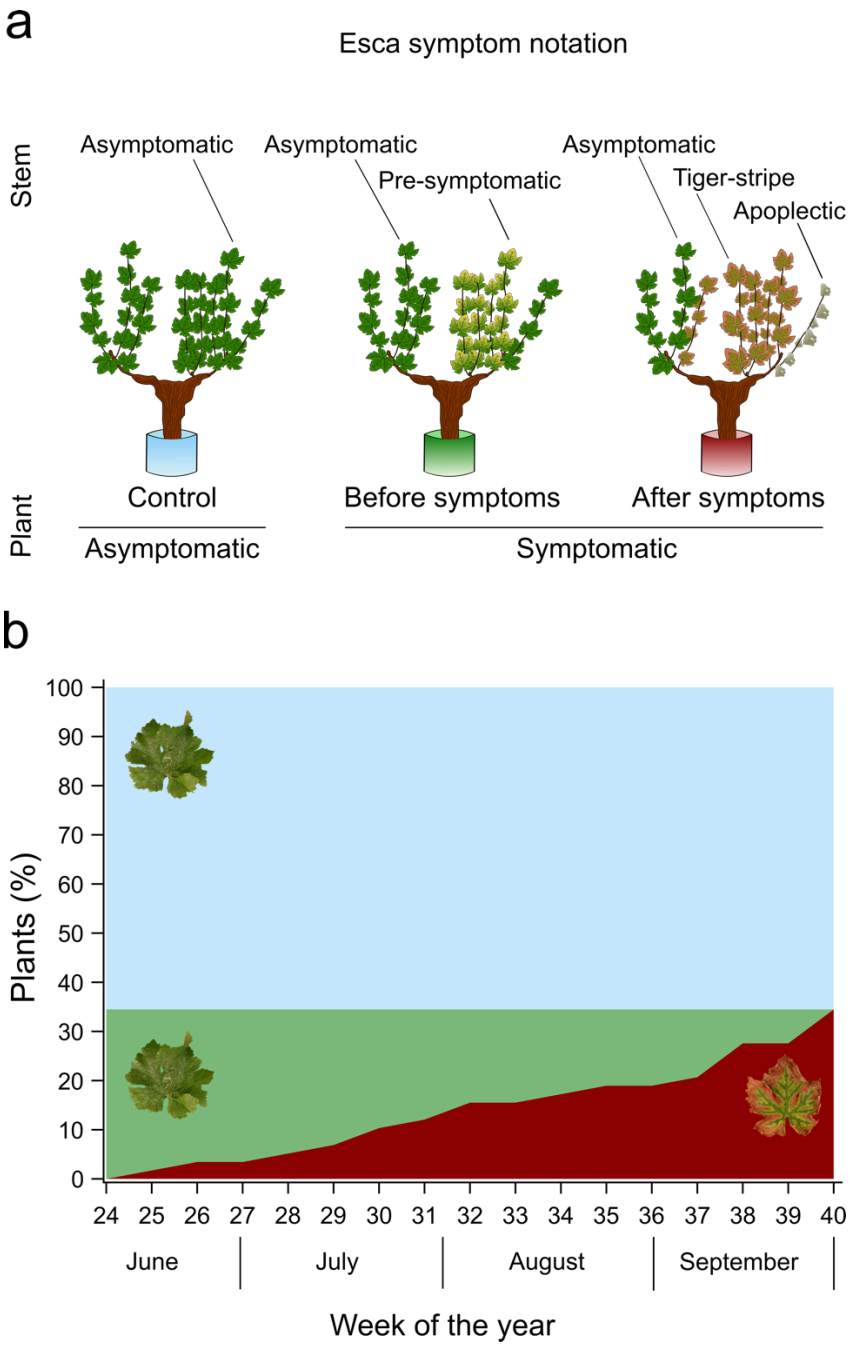
843 **Fig. S3**. Vessel density and percentage of occluded vessels in tiger-stripe stems for different
844 vessel diameter classes.

845 **Fig. S4**. Relationships between k_s and k_{th} in each stem symptom category.

846 **Table S1**. Effect of year of uprooting, internode analyzed, and sampling date on k_s and k_{th} in
847 control stems.

848 **Table S2.** Calculated theoretical hydraulic conductivity (k_{th} %), and hydraulic conductivity loss
849 (PLC %) from X-ray microCT volumes of intact grapevine stems.

850 **FIGURES**



851

852 **Fig. 1 (a-b)**. Representation of esca symptom notation during the experimental season. (**a**)
853 Single stems could be noted as esca asymptomatic, pre-symptomatic, tiger-stripe, or apoplectic.
854 Whole plants have been noted as control (asymptomatic from June to October) or symptomatic
855 (with tiger-stripe symptoms at the end of the season). (**b**) Proportion of plants in each symptom
856 category over the experimental season (n=58). The blue area corresponds to control plants,
857 green area to esca symptomatic plants before symptom appearance, and red area to esca
858 symptomatic plants.

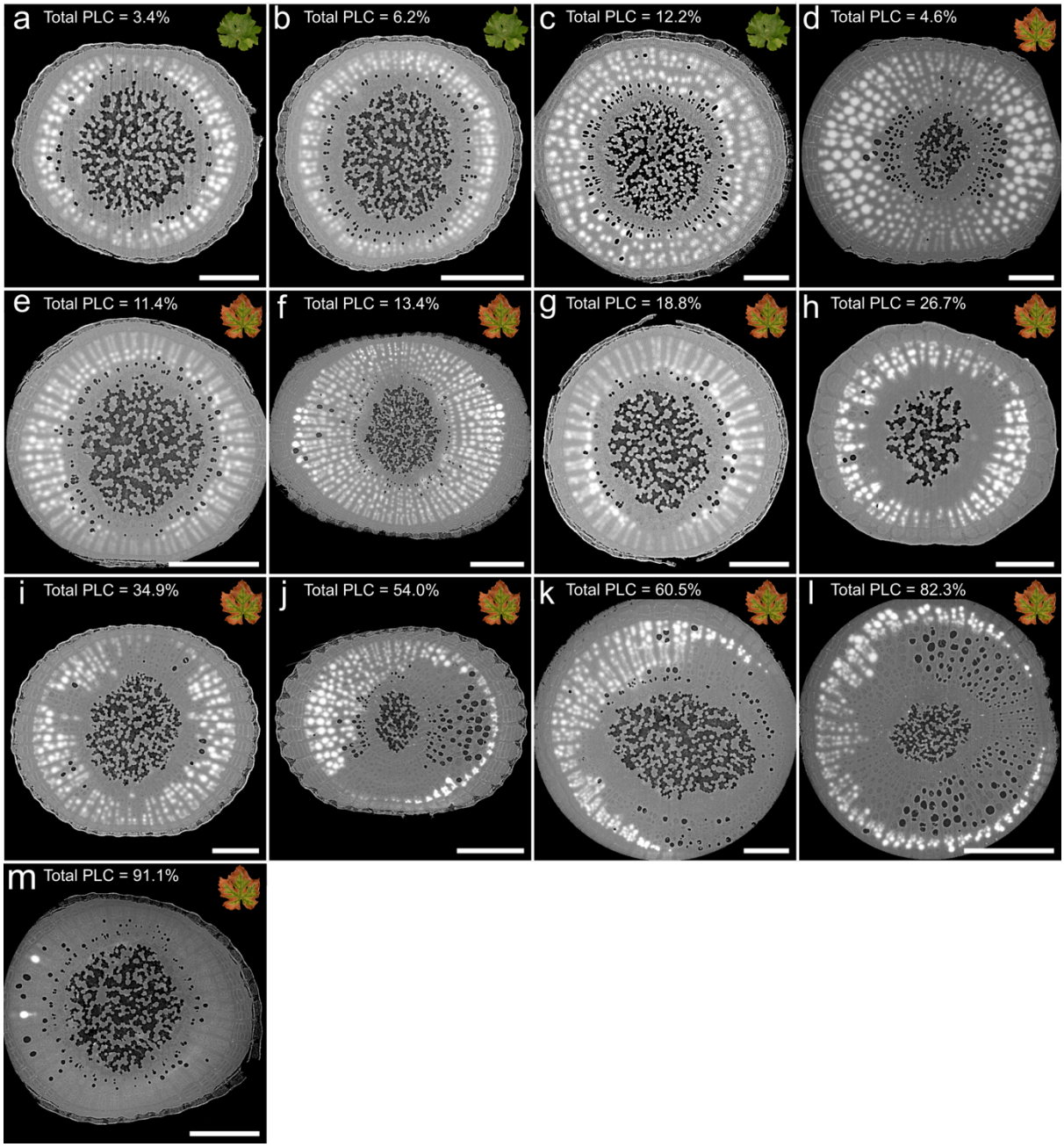


Fig. 2 (a-m). Two-dimensional reconstruction of cross-sections from X-ray microCT volumes of grapevine stems. Each panel represents a cross section of different stems for control (**a-c**) and esca symptomatic (**d-m**) plants. Iohexol appears white bright in functional vessels; air-filled vessels (i.e. native PLC) appear black; occluded vessels (i.e. occlusion PLC) appear grey. Total PLC (i.e. native PLC plus occlusion PLC) values are given for the presented samples. Scale bars = 1000µm.

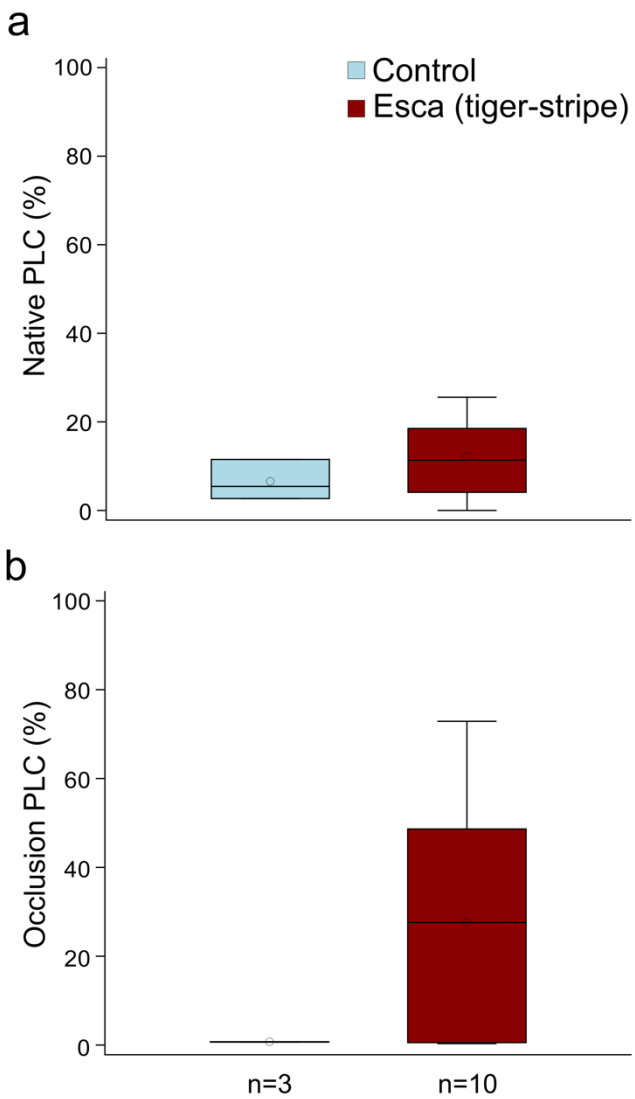


Fig. 3 (a-b). **(a)** Mean values of native PLC in control (blue) and esca tiger-stripe (red) stems of grapevine plants using X-ray microCT imaging. Differences were not significant (n=13, $F_{1,9}$=0.07, P=0.79). **(b)** Mean values of occlusion PLC in control (blue) and esca tiger-stripe (red) stems of grapevine plants using X-ray microCT imaging. Differences were not significant (n=13, $F_{1,9}$=0.33, P=0.58). Boxes and bars show the median, quartiles and extreme values, circles show mean values. N represents the sample size (number of analyzed stems) for each group.

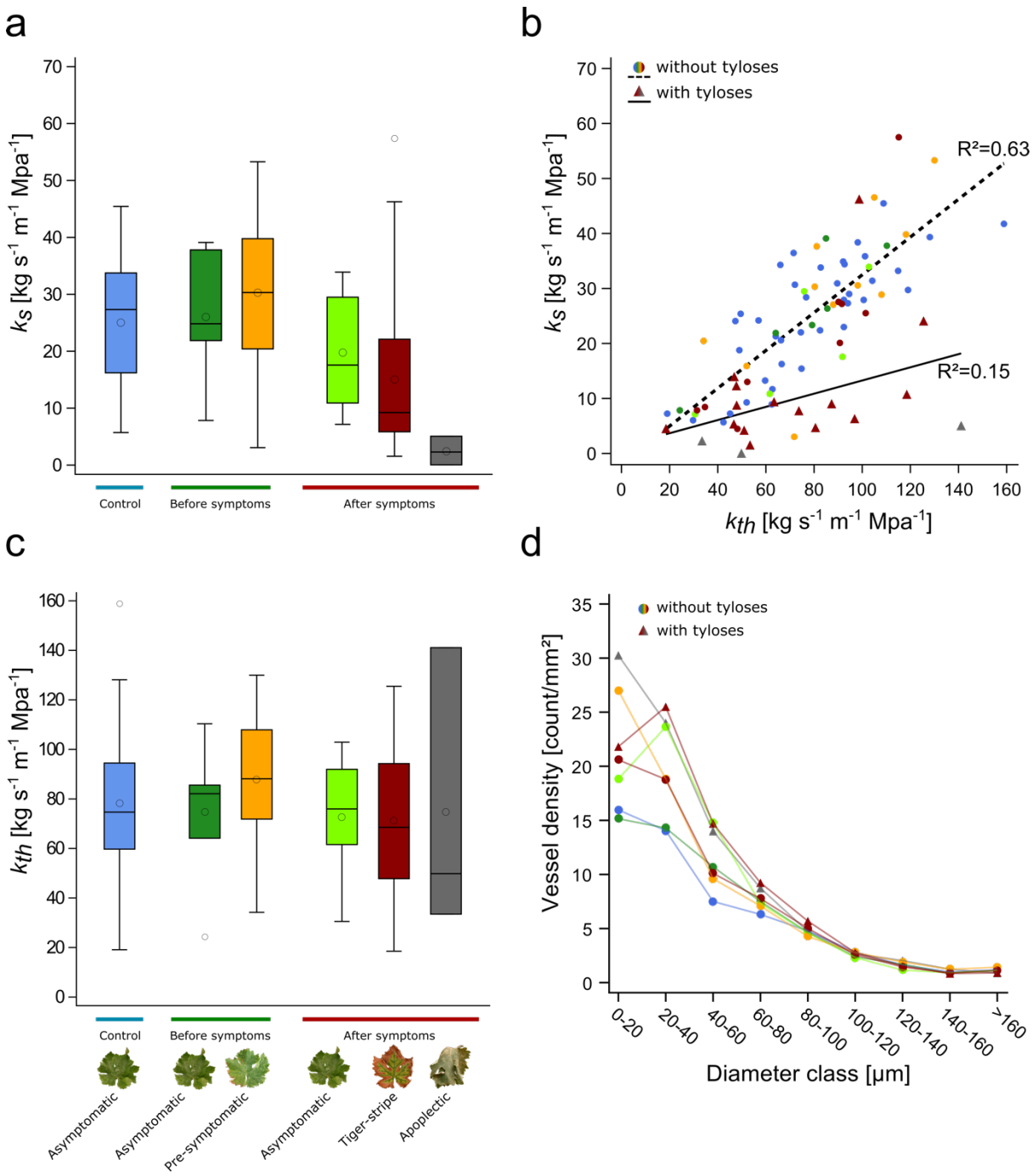


Fig. 4 (a-d). Relationships between specific stem hydraulic conductivity (k_s), theoretical stem hydraulic conductivity (k_{th}), and vessel density in control and esca symptomatic grapevine plants. **(a)** k_s values for control (blue); asymptomatic (dark green) and pre-symptomatic (yellow) stems in plants before symptom appearance; asymptomatic (light green), tiger-stripe (red), and apoplectic (grey) stems in plants after symptom appearance, differences were not significant (n=88, $F_{5,45}$=1.30 P=0.28). Boxes and bars show the median, quartiles and extreme values, circles within boxes correspond to means, and circles outside boxes to outlier values. **(b)** Relationships between k_s and k_{th}. Symbols represent the absence (circles) or presence

885 (triangles) of tyloses in xylem vessels. Colors represent esca symptomatology (as in panel a).
886 The dashed line represents the regression for stems in which no tyloses were observed in xylem
887 vessels, and the solid line represents the regression for samples with tyloses. R^2 for the
888 regression lines are indicated (see Table 2 and Fig. S4 for detailed analyses). **(c)** k_{th} values for
889 the different stem categories as presented in panel a. Differences were not significant (n=88,
890 $F_{5,45}$=0.58, P=0.71). **(d)** Relationships between mean values of xylem vessel density and their
891 diameters. Differences in total vessel density and in vessel size distributions were not
892 significant (n=88, $F_{6,45}$=0.77, P=0.60; n=792 (88 samples for 9 vessel classes), $F_{48,693}$=1.19,
893 P=0.18). Colors and markers are the same as panel b.

894 # TABLES

895

896 **Table 1**. Esca leaf symptom observations over the experimental season on *Vitis vinifera* cv
897 Sauvignon blanc.

Symptom notation before 2019 / **Symptom notation in 2019**	**All plants**	**Previously asymptomatic (pA)**	**Previously symptomatic (pS)**
Esca-symptomatic	35 % (20/58)	30 % (6/20)	37 % (14/38)
Control-asymptomatic	65 % (38/58)	70 % (14/20)	63 % (24/38)

898 Plants are grouped by their symptom history: previously asymptomatic (pA, plants that have
899 never expressed leaf symptoms between 2012 and 2018) and previously symptomatic (pS,
900 plants that have expressed leaf symptoms at least once since 2012). Ratios present the number
901 of plants in each symptom category (esca-symptomatic or control-asymptomatic) over the total
902 number of plants of the category.

903 **Table 2**. Values for specific stem hydraulic conductivity (k_s), theoretical stem hydraulic
904 conductivity (k_{th}) and equations of regression lines between k_s and k_{th} for control and esca
905 symptomatic stems.

Tyloses	Esca	k_s [kg s^{-1} m^{-1} Mpa^{-1}]	k_{th} [kg s^{-1} m^{-1} Mpa^{-1}]	n (stem - plant)	Regression
Absence	Control	24.97 ± 1.72	78.36 ± 4.51	39 - 23	k_s = 0.3 x k_{th} + 1.6 R^2=0.61 P<0.0001
	Asymptomatic before symptoms	26.04 ± 4.71	74.75 ± 11.80	6 - 6	k_s = 0.36 x k_{th} - 0.94 R^2=0.82 P=0.013
	Pre-symptomatic	30.32 ± 4.26	87.88 ± 8.54	11 - 7	k_s = 0.37 x k_{th} - 1.9 R^2=0.54 P=0.010
	Asymptomatic after symptoms	19.80 ± 5.18	72.58 ± 12.64	5 - 2	k_s = 0.33 x k_{th} - 4 R^2=0.64 P=0.104
	Esca (tiger-stripe)	21.29 ± 5.40	72.85 ± 10.41	9 - 5	k_s = 0.45 x k_{th} - 11.26 R^2=0.74 P=0.003
Presence	Esca (tiger-stripe)	11.27 ± 2.86	70.44 ± 7.81	15 - 5	k_s = 0.17 x k_{th} -0.84 R^2=0.22 P=0.077
	Esca (apoplectic)	2.47 ± 1.45	74.80 ± 33.48	3 - 2	k_s = 0.04 x k_{th} - 0.2 R^2=0.68 P=0.385
Absence	All	25.06 ± 1.46	78.42 ± 3.37	70 - 37	k_s = 0.34 x k_{th} - 1.90 R^2=0.63 P<0.0001
Presence	All	9.81 ± 2.51	71.16 ± 8.00	18 - 7	k_s = 0.12 x k_{th} - 1.28 R^2=0.15 P=0.117

906 Values represent mean ± SE. n = sample size, (including the number of analyzed stems and-
907 number of analyzed plants, respectively). See text and Fig. 4 for statistical analysis. A detailed
908 esca symptom notation is provided in Fig. 1a. Bivariate plots of each regression are presented
909 in Fig. S4.

910 **Table 3**. Long-term impact of symptom presence (i.e. comparing plants with different disease
911 history record) in control plants on specific stem hydraulic conductivity (k_s), theoretical stem
912 hydraulic conductivity (k_{th}), stem total vessel density, and amount of *Phaeomoniella*
913 *chlamydospora* and *Phaeoacremonium minimum* DNA in trunks.
914

	Previously asymptomatic (pA)	**Previously asymptomatic (pS)**	**Type III Tests of Fixed Effects (pA vs pS)**
k_s [kg s^{-1} m^{-1} Mpa^{-1}]	23.76 ± 2.30	26.54 ± 2.61	n=39, $F_{1,16}$=1.19, P=0.29
k_{th} [kg s^{-1} m^{-1} Mpa^{-1}]	72.22 ± 4.85	86.30 ± 7.98	n=39, $F_{1,16}$=3.01, P=0.10
total vessel density [count mm^{-2}]	57.28 ± 4.03	52.61 ± 3.25	n=39, $F_{1,16}$=0.72, P=0.41
P. chlamydospora [pg ng^{-1}]	6.14 ± 1.90	10.15 ± 3.41	**n=13, $F_{1,11}$=5900.06, P<0.0001**
P. minimum [pg ng^{-1}]	9.27 ± 6.97	26.40 ± 13.83	**n=13, $F_{1,11}$=51014, P<0.0001**

915 Values represent means ± SE. Pathogen quantification was estimated as: pg fungal DNA ng^{-1}
916 total DNA. Statistical tests used are individual generalized linear mixed models to compare pA
917 vs pS plants (fixed effect) with the individual plants entered as a random effect in the models
918 and the year of uprooting as a co-variable (fixed effect). Statistically significant results
919 (P<0.05) are shown in bold.

920 **Table 4.** Quantification by qPCR of *Phaeomoniella chlamydospora* and *Phaeoacremonium*
921 *minimum* DNA in stems and trunks of different esca symptomatology.

Organ	Esca	n	*P. chlamydospora* [pg ng^{-1}]	*P. minimum* [pg ng^{-1}]
Stem	Control	8	0	0
	Pre-symptomatic	3	0	0
	Asymptomatic (after symptoms)	3	0	0
	Tiger-stripe (without tyloses)	4	0	0
	Tiger-stripe (with tyloses)	8	0	0
	Apoplectic	2	0	0
Trunk	Control	13	7.37 ± 1.67 (12/13)*	14.54 ± 6.51 (12/13)*
	Symptomatic	7	15.80 ± 3.12 (7/7)*	23.90 ± 8.82 (7/7)*

* Number of samples positive for the pathogen over the total number of analyzed samples.

922 Pathogen quantification was estimated as: pg fungal DNA per ng total DNA.Values represent
923 means ± SE, n = sample size. Trunks of symptomatic plants presented higher amount of both
924 *P. chlamydospora* and *P. minimum*, compared to control (n=20, $F_{1,18}$=29806.11.25, P<0.0001
925 and n=20, $F_{1,18}$=21925.4, P<0.0001, respectively). See text for statistical methods.