Supplementary data

Title: Esca grapevine disease involves leaf hydraulic failure and represents a unique premature senescence process.

The following Supplementary Data are available for this article:

Table S1. Effect of esca leaf symptoms on the presence of occluded vessels in *Vitis vinifera*.

Table S2. Linear relationships between the percentage of occluded vessels and esca symptom severity (expressed in percentage of green tissue per symptomatic leaf, control leaves were excluded from the analysis) for each *Vitis vinifera* cultivar.

Figure S1. Pictures of the three senescence types from a white cultivar (Chenin, A, C, E, G) and a red (Castet, B, D, F, H) *Vitis vinifera* cultivar.

Figure S2. Relationship between percentage of xylem vessels with crystals and vessels with occlusion (examples in Fig. 1) for each sample in *Vitis vinifera*.

Figure S3. Occluded vessels [%] from different cultivars and different countries in *Vitis vinifera* midribs collected from leaves with (from light red to dark red) and without (blue, all cultivars combined) esca symptoms.

Supplemental Discussion SD1. Crystals in xylem vessels.

Supplementary References

Table S1. Effect of esca leaf symptoms on the presence of occluded vessels vessels in *Vitis vinifera*. Statistical tests used are individual generalized linear models to compare control versus esca symptomatic plants (fixed effect). Statistically significant results (P<0.05) are shown in bold

Country – cultivar	Sample size (Control - Esca)	*DoF*	*F*	*P*
France – Sauvignon blanc	(3 - 5)	**1,6**	**19.16**	**0.005**
France – Mourvedre	(3 - 2)	1,3	3.67	0.15
France – Castet	(5 - 5)	1,8	1.64	0.24
France – Chenin	(2 - 5)	**1,5**	**24.43**	**0.004**
France – Sangiovese	(4 - 5)	**1,7**	**29.22**	**0.001**
Italy – Sauvignon blanc	(3 - 4)	**1.5**	**1123.2**	**<0.0001**
Italy – Sangiovese	(3 - 5)	**1,6**	**200.4**	**<0.0001**
California – Sauvignon blanc	(8 - 7)	**1,13**	**20.7**	**0.0005**
Spain – Castet	(3 - 5)	**1,6**	**17.06**	**0.006**
Spain – Grenache	(1 - 5)	Absence of tyloses both in control and esca		
Spain – Tempranillo	(3 - 5)	**1,2**	**6.65**	**0.04**
Spain - Tempranillo blanco	(1 - 3)	**1,6**	**477.7**	**0.002**

Table S2. Linear relationships between the percentage of occluded vessels and esca symptom severity (expressed in percentage of green tissue per symptomatic leaf, control leaves were excluded from the analysis) for each *Vitis vinifera* cultivar.

Cultivar (symptomatic)	**Regression curve**	**R^2**	***P***
All cultivars	y = -0.1x + 26.39	0.017	0.42
Grenache	flat (0 occlusion)		
Tempranillo	y = 0.25x + 2.40	0.26	0.38
Mourvedre	2 samples		
Castet	y = 0.17x + 7.58	0.12	0.32
Sauvignon blanc	y = -0.37x + 47.56	0.22	0.07
Chenin	y = -1.51x + 92.13	0.35	0.29
Sangiovese	y = -0.68x + 64.76	0.56	0.01
Tempranillo blanco	y = -0.41x + 64.39	0.31	0.63

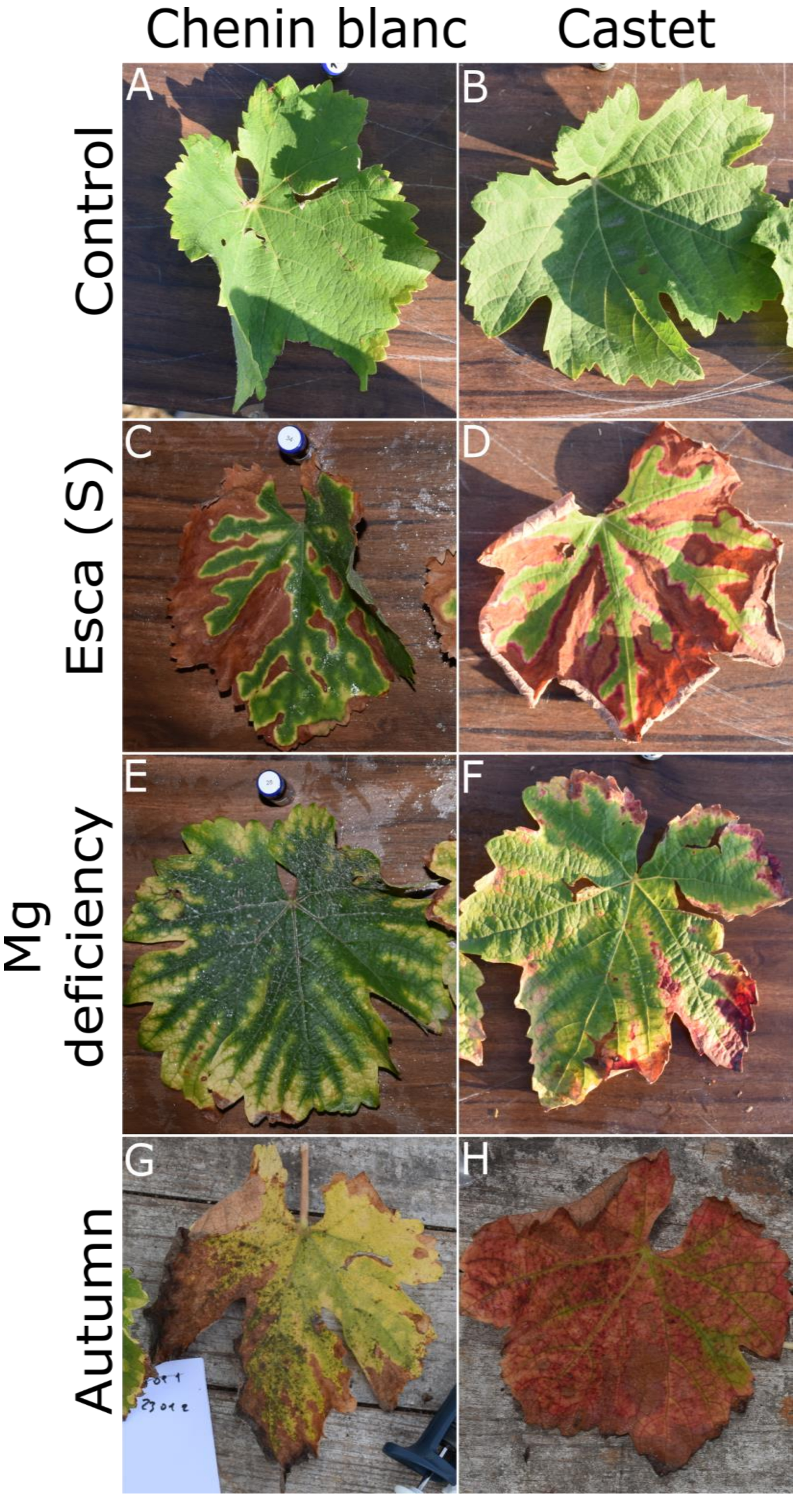

Figure S1. Pictures of the three senescence types from a white cultivar (Chenin, A, C, E, G) and a red (Castet, B, D, F, H) *Vitis vinifera* cultivar. The phenotype is visibly similar at the leaf level (discolorations and scorched tissue), but it can be distinguished by their distribution in the canopy and timing of development in the field, as described in material and methods. (**A**, **B**) Healthy (control) leaves in August. (**C**, **D**) Esca symptomatic leaves in August. (**E**, **F**) Magnesium-deficient leaves in August. (**G**, **H**) Autumn-senescent leaves in October.

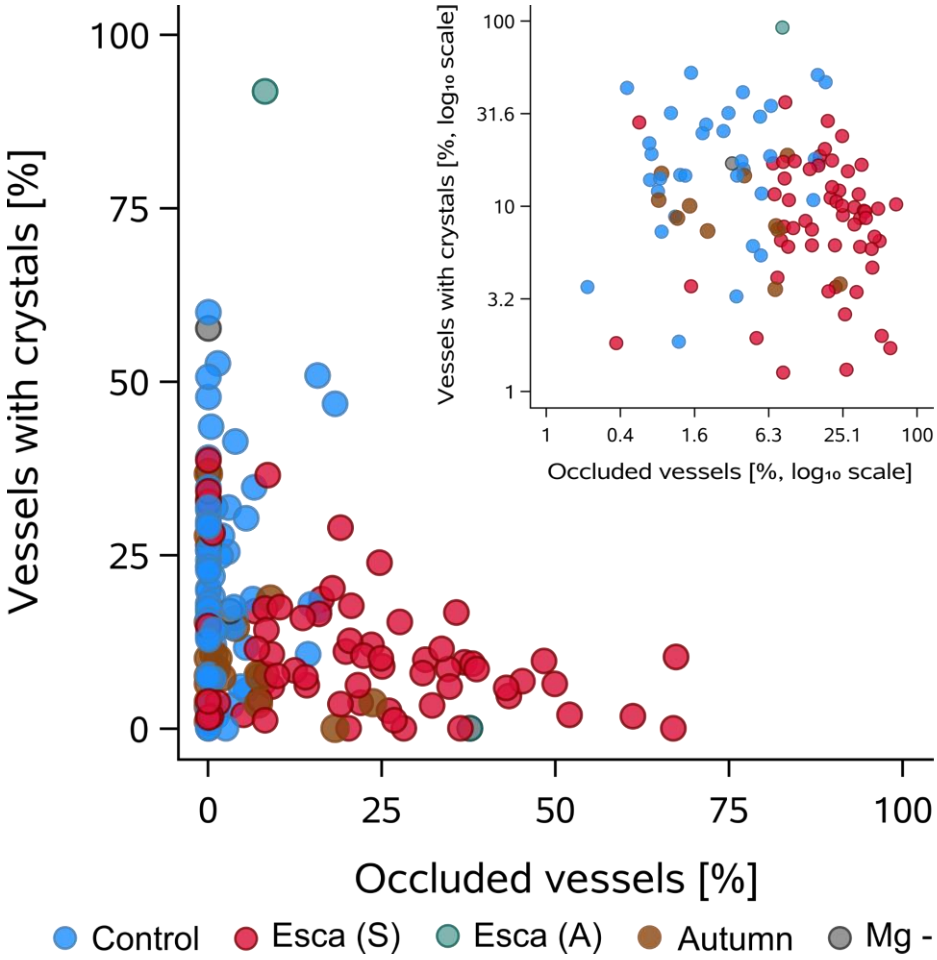

Figure S2. Relationship between percentage of xylem vessels with crystals and vessels with occlusion (examples in Fig. 1) for each sample in *Vitis vinifera*. Symbols represent leaf samples (blue for control, red for esca symptomatic, green for asymptomatic leaves in symptomatic plants, brown for leaves in autumn, and gray for leaves under magnesium deficiency). In the inset the same relationship is shown in log scale. The relationship was significant for linear (P<0.0001, R^2=0.1) regression, and not significant for logarithmic (P=0.06, R^2=0.03) regression.

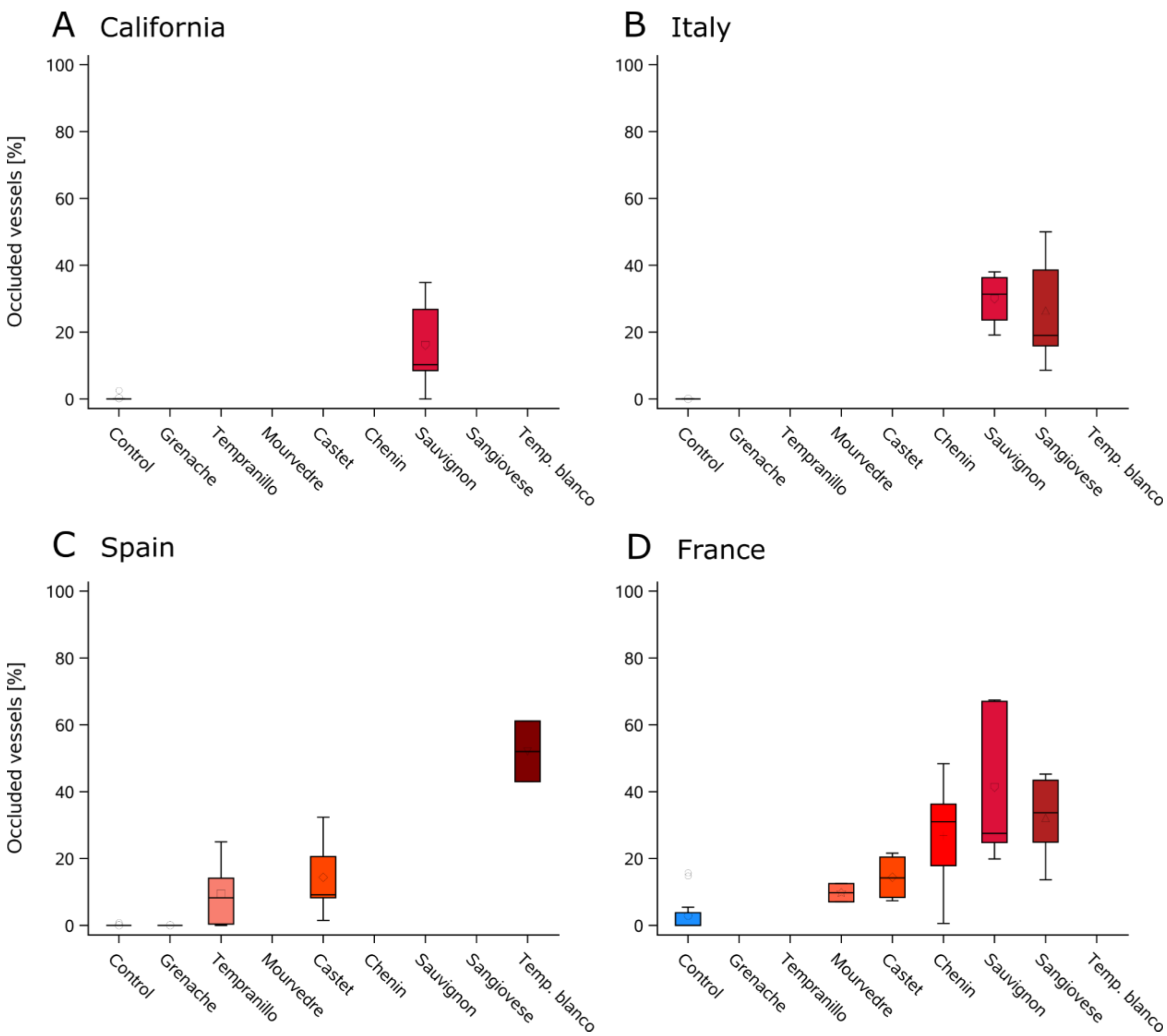

Figure S3. Occluded vessels [%] from different cultivars and different countries in *Vitis vinifera* midribs collected from leaves with (from light red to dark red) and without (blue, all cultivars combined) esca symptoms. Boxes and bars show the median, quartiles, and extreme values. Symbols inside boxes show mean values; symbols outside boxes show outliers. (**A**) Occlusion levels in leaves from California ($F_{1,13}$=20.7, P=0.0005). (**B**) Occlusion levels in leaves from Italy, where cultivar differences were significant ($F_{1,13}$=715.1, $P<0.0001$). (**C**) Occlusion levels in leaves from Spain, where cultivar differences were significant ($F_{1,24}$=9.42, P=0.005). (**D**) Occlusion levels in leaves from France, where cultivar differences were significant ($F_{1,37}$=46.92, $P<0.0001$).

Supplemental Discussion SD1.

Crystals in xylem vessels

We regularly observed crystals inside xylem vessels. In the literature, when crystals are observed in leaves, authors usually refer to oxalate calcium, which is formed to regulate calcium bulk pressure, defense to herbivores, or metal detoxification (Franceschi and Nakata, 2005). Oxalate calcium crystals are found in leaves from different species (Somavilla et al., 2014) and this is also the case for grapevine (Jáuregui-Zúñiga et al., 2003, Şeker et al., 2016). However, to our knowledge, calcium oxalate was observed in other studies in parenchyma cells, but never inside xylem vessels. One study found crystals in xylem vessels contaminated by fungi (Chattaway, 1952, in different woody species). More recently, Sun et al. (2013) found prismatic crystals in grapevine stems (in ~3% of vessels) following *Xylella fastidiosa* inoculations. In our case, we found crystals (both prismatic and druse) in 15% of vessels (on average) in midribs, regardless of leaf phenotype. As such, we considered them as non-influential to xylem water transport. Confirming this result, Calzarano et al. (2014) found that druse oxalate calcium crystals were present in asymptomatic leaves, whereas they were absent from esca symptomatic leaves. However, other studies should investigate the chemical nature of these crystals, to identify their role in plant physiology and whether they are artifacts of sample preparation (e.g. fixation, impregnation, or inclusion protocols).

Supplementary References

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