

Supporting information for:

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

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Method S1. Image analysis of microCT scans

In the high energy scans recorded at 33.3 keV X-ray beam, iodhexol appears bright white but its contrast can sometimes impede the clear limit of the vessel lumen. Therefore, all vessel diameters were recorded on the scan recorded at low energy (33.1 keV X-ray beam), then the distinction of occluded from iodhexol-filled vessels was done on the high energy scan (as done by Bortolami et al. 2019). The theoretical hydraulic conductivity of each vessel (k_{vessel}) [$\text{kg m Mpa}^{-1} \text{ s}^{-1}$] was calculated using the Hagen-Poiseuille equation:

$$k_{vessel} = (\pi * \theta^4 * \rho) / (128 * \eta)$$

Where: θ is the equivalent circle diameter [m], ρ the density of water [998.2 kg m^{-3} at 20°C], and η the viscosity of water [$1.002 * 10^{-9} \text{ MPa s}$ at 20°C]. The percentage theoretical conductivity loss given by native air embolism (native PLC) was calculated by the ratio between the hydraulic conductivity of air-filled vessels and the whole-stem hydraulic conductivity:

$$\text{Native PLC (\%)} = 100 * (\sum k_{\text{air-filled vessels}}) / (\sum k_{\text{all vessels}})$$

The percentage theoretical conductivity loss given by occlusions (occlusion PLC) was calculated by the ratio between occluded (plus partially occluded) vessels and the hydraulic conductance of the stem:

$$\text{Occlusion PLC (\%)} = 100 * (\sum k_{\text{occluded vessels}} + \sum k_{\text{partially occluded vessels}}) / (\sum k_{\text{all vessels}})$$

The total percentage theoretical conductivity (total PLC) was obtained by summing native PLC with occlusion PLC in each sample. As the first ring of xylem vessels (i.e. protoxylem) was always not functional ($>90\%$ PLC), both in control and tiger-stripe stems, it was removed from the analysis.

Method S2. Specific stem hydraulic conductivity (k_s) measurements

Stem hydraulic conductivity measurements were performed on one internode of $>1.5\text{m}$ long one-year old stems, following Torres-Ruiz et al. (2012) gravity method. In the early morning, each stem was cut at the base under water to avoid air entrance in the stem, maintained under water and brought to the laboratory. Hydraulic conductivity measurements were always done before noon, in order to minimize the delay (never more than four hours) from the cut to the measure. In the laboratory, a representative internode between the 4th to the 10th internode from the base was cut underwater with a clean razor blade, the ends were wrapped in tape, and the

internode was connected to a pipe system. A flow of 20 mM KCl solution passed through the sample from a reservoir to a precision electronic balance (AS220.R2, RADWAG, Radom, PL) recording the weight every 5 seconds using the WinWedge v3 5.0 software (TAL Technologies, Philadelphia, PA, USA). The solution was passed through the stem at four increasing pressures (ranging from 0.001 to 0.005 MPa), controlled by raising the source height. The average flow for each pressure step was determined after stabilization at a steady-state as the average of 10-15 measures. Hydraulic conductance, k [$\text{kg s}^{-1} \text{MPa}^{-1}$] was obtained by the slope generated by the flow and the corresponding pressure. The linear relationship between flow and pressure obtained were always characterized by $R^2 > 0.97$.

Stem specific hydraulic conductivity, k_s [$\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$], was calculated as follows:

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

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

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

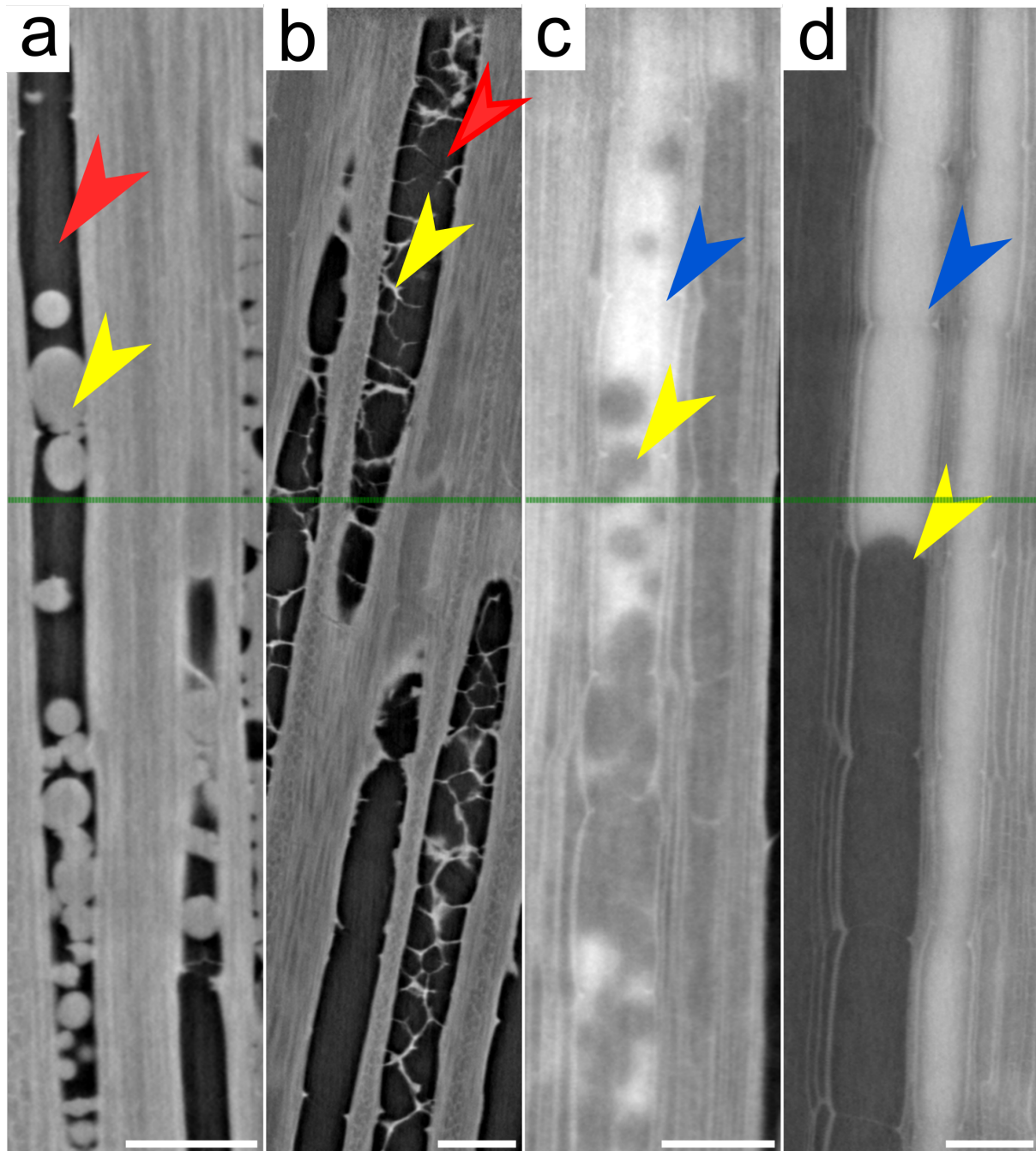


Fig. S1 (a-d). Two-dimensional reconstruction of longitudinal cross sections from X-ray microCT volumes of grapevine stems, examples of apparently air-filled (a, b; red arrowheads), and apparently functional (c, d; blue arrowheads) vessels. Tyloses can only partially occlude the vessels (a, c, d, yellow arrowheads), or only the tylose walls are present without cellular content (b, yellow arrowheads). If only transversal cross sections (e.g. green lines) are analyzed, those vessels could be mistakenly considered as non-occluded. Scale bars = 200 μ m.

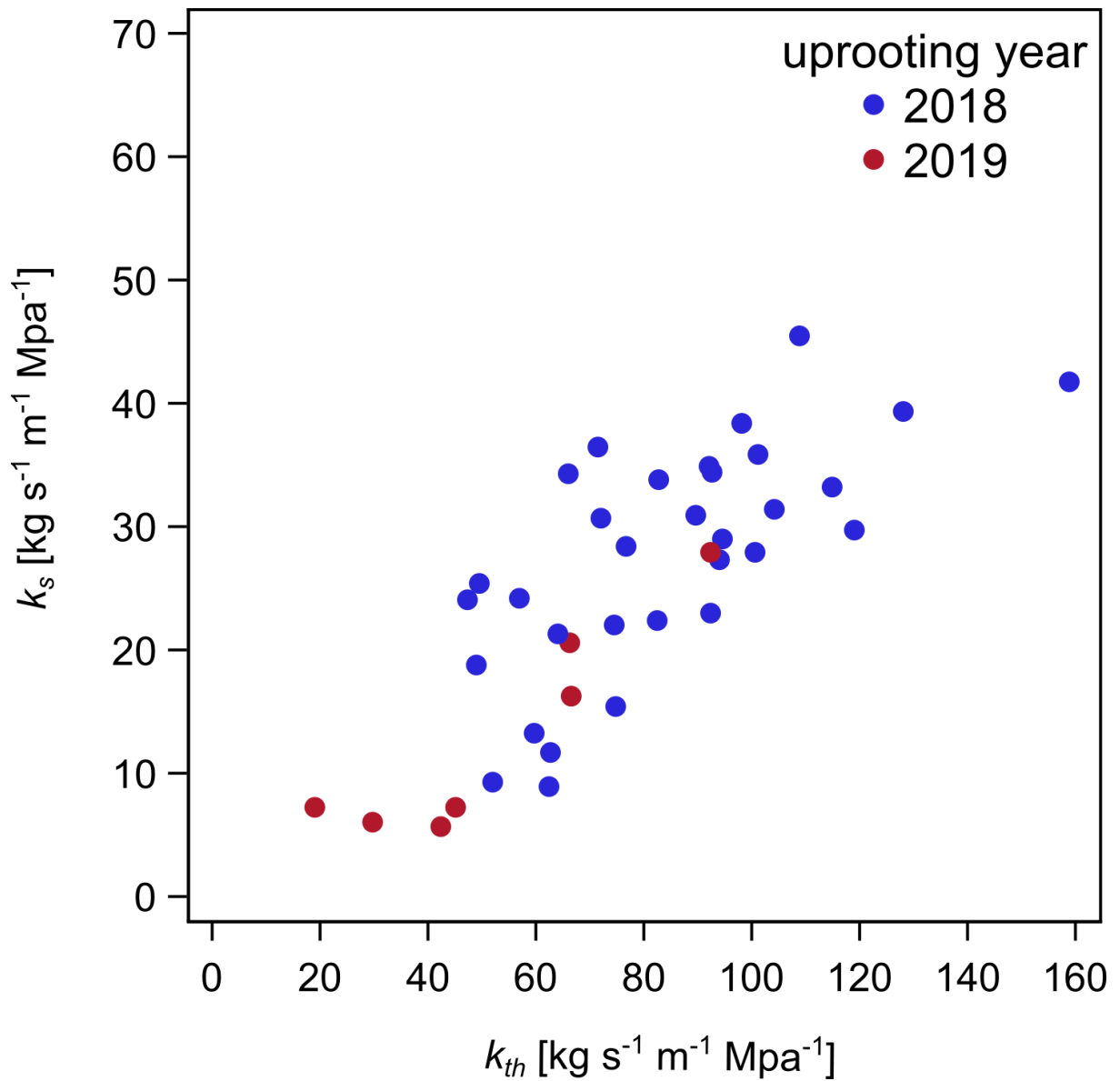


Fig. S2. Relationship between k_s and k_{th} in control plants. Blue dots represent plants uprooted in 2018 and red dots represent plants uprooted in 2019.

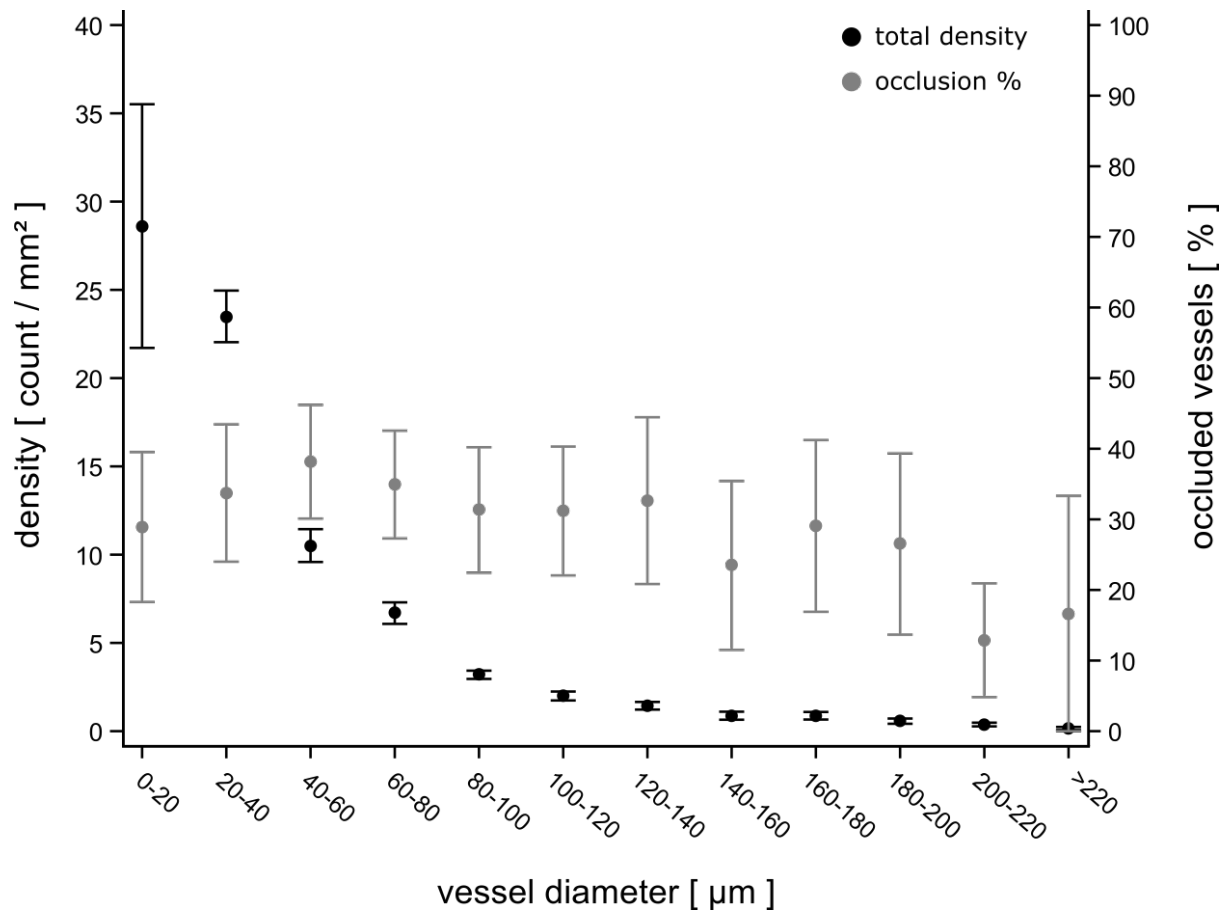


Fig. S3. Mean vessel density (black circles) in tiger-stripe stems for different vessel diameter classes and mean percentage of occluded vessels (grey circles) in each class from X-ray microCT imaging analysis. Error bars represent standard errors.

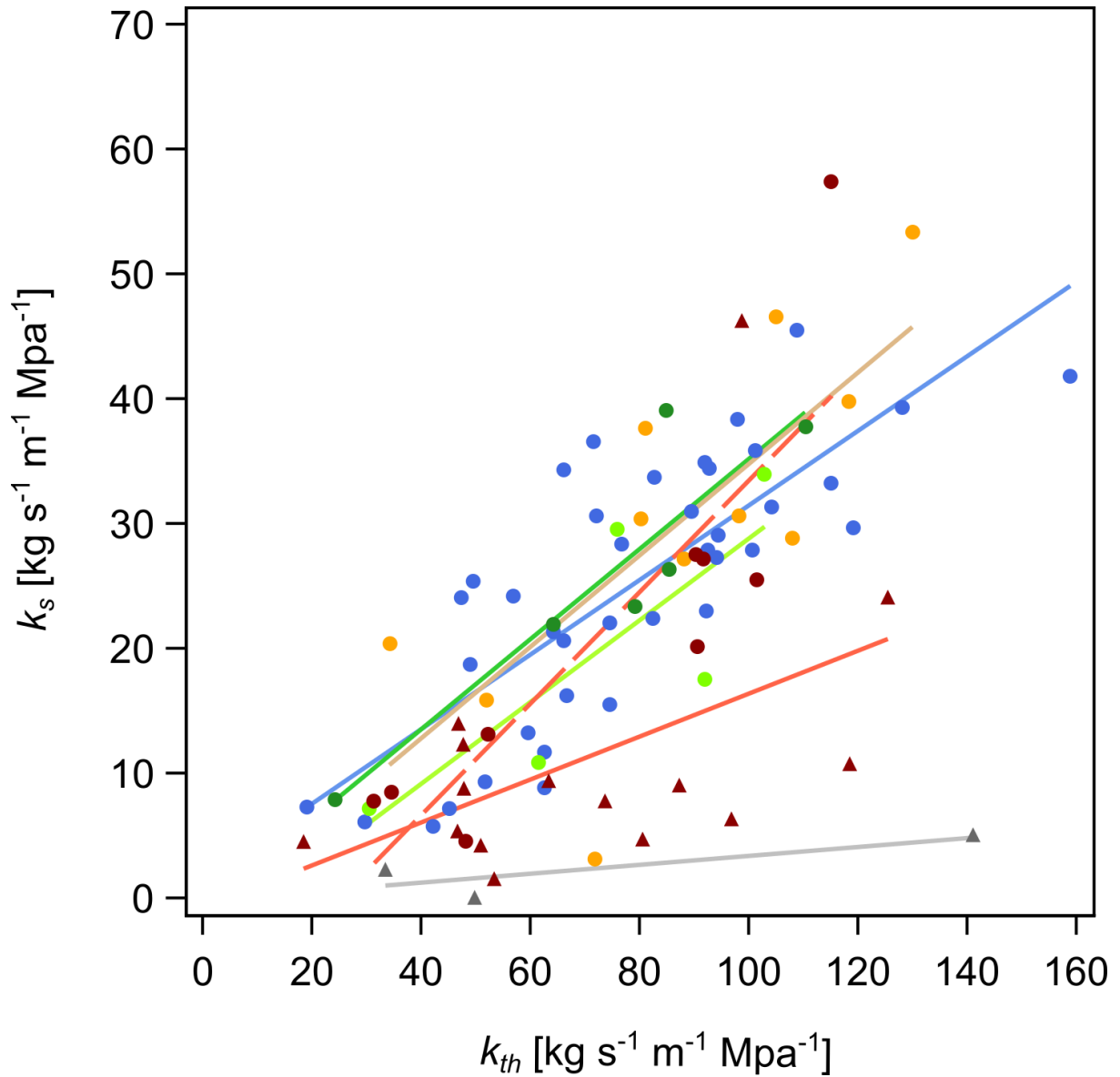


Fig. S4. Relationships between k_s and k_{th} . Blue symbols represent measurements in control stems; dark green symbols represent asymptomatic stems in plants before symptom appearance; yellow symbols represent pre-symptomatic stems in plants before symptom appearance; light green symbols represent asymptomatic stems in plants after symptom appearance; red circles and dashed red line tiger-stripe stems without tyloses; red triangles and solid red line tiger-stripe stems with tyloses; grey symbols represent apoplectic stems. R^2 and equations of regression lines are presented in Table 2.

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

| Fixed effects | k_s (only control plants, n=39) | k_{th} (only control plants, n=39) |
|-------------------|---|--|
| Year of uprooting | F_{1,16} = 10.31 P = 0.006 | F_{1,16} = 9.38 P = 0.007 |
| Internode | F _{4,12} = 2.15 P = 0.14 | F _{4,12} = 2.22 P = 0.13 |
| Sampling date | F _{7,9} = 1.77 P = 0.21 | F _{7,9} = 1.53 P = 0.27 |

Individual generalized linear mixed models with the individual plants entered as a random effect in the models. Statistically significant results (P<0.05) are shown in bold.

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

| Stems | n | Functional k_{th} | Native PLC | Occlusion PLC |
|---------------------|----|---------------------|--------------|---------------|
| Control | 3 | 92.75 ± 2.60 | 6.54 ± 2.61 | 0.71 ± 0.02 |
| Esca (tiger-stripe) | 10 | 60.22 ± 9.70 | 12.25 ± 2.87 | 27.53 ± 8.24 |

Values are means ± standard error, n = sample size