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Marylou Mantova, Paulo Eduardo Menezes-Silva, Eric Badel, Hervé Cochard,
Jose Manuel Torres Ruiz

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The interplay of hydraulic failure and cell vitality explains tree capacity to recover from drought

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4 **recover from drought**
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10 4 Marylou Mantova¹, Paulo E. Menezes-Silva², Eric Badel¹, Hervé Cochard¹, José M. Torres-
11 Ruiz^{1,*}
12
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14 6

15
16 7 ¹Université Clermont Auvergne, INRAE, PIAF, 63000 Clermont-Ferrand, France

17
18 8 ²Laboratório de Fisiologia Vegetal, Instituto Federal de Educação, Ciência e Tecnologia Goiano, IF
19 Goiano, 75901970 Rio Verde, Brasil
20
21

22 10

23
24 11 **Correspondence**

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26
27 12 *Corresponding author,

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29 13 e-mail : torresruizjm@gmail.com
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3 15 **Abstract**
4

5 16 Global climatic models predict an increment in the frequency and intensity of drought events, which
6 17 have important consequences on forest dieback. However, the mechanisms leading to tree mortality
7 18 under drought conditions and the physiological thresholds for recovery are not totally understood yet.
8 19 This study aimed to identify what are the key physiological traits that determine the tree capacity to
9 20 recover from drought. Individuals of a conifer (*Pseudotsuga menziesii* M.) and an angiosperm (*Prunus*
10 21 *lusitanica* L.) species were exposed to drought and their ability to recover after rehydration monitored.
11 22 Results showed that the actual thresholds used for recovery from drought based on percentage loss of
12 23 conductance (PLC) (i.e. 50% for conifers, 88% for angiosperms) do not provide accurate insights about
13 24 the tree capacity for surviving extreme drought events. On the contrary, differences in stem relative
14 25 water content (RWC_{Stem}) and the level of electrolytes leakage (EL) were directly related to the capacity
15 26 of the trees to recover from drought. This was the case for the conifer species, *P. menziesii*, for which
16 27 higher RWC_{Stem} and lower EL values were related to the recovery capacity. Even if results showed a
17 28 similar trend for the angiosperm *P. lusitanica* as for the conifers, differences between the two traits were
18 29 much more subtle and did not allow an accurate differentiation between trees able to recover and those
19 30 that were not. RWC_{Stem} and EL could work as indicators of tree capacity to recover from drought for
20 31 conifers but more studies are required to confirm this observation for angiosperms.
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33 Introduction

34 Forests represent ca. 30% of the global continental surface (FAO 2006) and provide society with
35 several ecosystem services such as timber production, watershed protection (Allen et al. 2010), hosting
36 biodiversity (Trumbore et al. 2015), and carbon storage and its associated atmospheric feedbacks
37 (Reichstein et al. 2013). Due to the ongoing climate changes and global warming (IPCC 2014), not only
38 the frequency of heatwaves and drought events have increased in many areas worldwide but also their
39 duration and intensity (Allen et al. 2010). A recent data synthesis has suggested that the majority of
40 plant species converge to narrow hydraulic safety margins and thus are very susceptible to changes in
41 rainfall patterns (Choat et al. 2018). Therefore, these higher frequencies and increased severity have
42 exacerbated the occurrence of drought-induced tree mortality events (Keenan et al. 2013, Duan et al.
43 2014) and, consequently, forests dieback (Hosking and Hutcheson 1988, Lwanga 2003, Landmann and
44 Dreyer 2006).

45 Although the reduction in water availability can affect virtually all processes associated with
46 plant growth and development, drought-induced tree mortality events are commonly associated with
47 two main processes: carbon starvation and xylem hydraulic failure (McDowell et al. 2008). Under
48 prolonged mild drought conditions, trees partially close their stomata to reduce evapotranspiration and
49 hence the risk of xylem hydraulic failure. However, this stomatal closure constrains CO₂ diffusion in
50 leaves and can lead to an important depletion of the carbohydrate pools resulting in carbon starvation
51 (Hogg and Hurdle 1997, Buckley 2005, McDowell et al. 2008, Berry et al. 2010, Creek et al. 2020).
52 Even if carbon starvation and xylem hydraulic failure cannot be considered as mutually exclusive
53 processes, recent studies have shown that xylem hydraulic failure is the main cause of tree mortality
54 under severe drought (Urli et al. 2013, Salmon et al. 2015, Adams et al. 2017). Xylem hydraulic failure
55 occurs when the tension in the continuous columns of water that connect the roots with the leaves
56 through the xylem increases and, consequently, exacerbates the risk of cavitation (breakage of the water
57 column) (Tyree and Zimmermann 2002). This process is widely amplified as soil dries or when the
58 evaporative demand increases. Thus, under extreme drought conditions, as the percentage of cavitaded
59 conduits increases, the hydraulic conductance of the xylem decreases until the flow of water stops and
60 provokes the desiccation of the plant tissues, cells death and, finally, the death of the tree (McDowell et
61 al. 2008). This makes xylem vulnerability to cavitation one of the main physiological traits when
62 evaluating drought-induced mortality.

63 Even if the vulnerability to cavitation has been widely evaluated for an important amount of
64 species during the last decades (Delzon et al. 2010, Choat et al. 2012), the relationship between xylem
65 hydraulic failure and tree mortality has not been properly evaluated yet. It is known that P_{50} and P_{88} (i.e.
66 the xylem tension inducing 50 and 88% of loss of hydraulic conductance, respectively) are associated
67 with the capacity of the trees to recover from drought (Brodribb et al. 2010, Delzon and Cochard 2014,

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3 68 Sperry and Love 2015, Bolte et al. 2016), but the physiological causes of tree death under extreme
4 69 drought events remain unclear. Therefore, due to a lack of physiological thresholds to properly define
5 70 tree mortality both during or after a drought episode, P_{50} and P_{88} values, for conifers and angiosperms
6 71 respectively (Brodribb and Cochard 2009; Urli et al. 2013), are currently used as proxies for mortality
7 72 when e.g. modelling the trees' response to drought (Martin-StPaul et al. 2017). However, a recent study
8 73 by Hammond et al. (2019) reported that the appropriateness of P_{50} as an indicator of mortality for
9 74 conifers should be reconsidered as they defined a lethal threshold at 80% of loss of xylem hydraulic
10 75 conductivity in loblolly pines (*Pinus taeda* L.). Also, it has recently been reported that branch diameter
11 76 variations were revealing a point of no recovery in lavender species as plants were not able to recover
12 77 from drought once their elastic water storage localized in the bark were depleted (Lamacque et al. 2020).
13 78 These results have raised new questions about the role of xylem hydraulic failure in triggering tree
14 79 mortality or the minimum hydraulic functioning required for allowing trees to survive and recover from
15 80 drought.

16 81 The ability of the trees to recover after a drought event seems to be tightly related to their ability
17 82 to grow new xylem (Brodribb et al. 2010) and this ability must be intrinsically linked to their capacity
18 83 to maintain key living tissues alive in perennial organs, as the stem, that allow them to regrowth and
19 84 resprout in favourable conditions. Considering the tenet that xylem hydraulic failure should provoke the
20 85 complete desiccation of the cells and their consequent death leading to whole plant mortality (McDowell
21 86 et al. 2008), a focus on plant water status and its consequences on cell vitality seems necessary to
22 87 understand drought-induced mortality (Guadagno et al. 2017, Martinez-Vilalta et al. 2019). This,
23 88 therefore, highlights the relevance of relative water content (RWC), a direct measure of the plant water
24 89 status at cell level, as a potential candidate for assessing drought-induced tree mortality (Martinez-
25 90 Vilalta et al. 2019, Trueba et al. 2019). In addition, and considering that many studies have evinced how
26 91 low RWC values are linked to membrane dysfunction in plant cells (Wang et al. 2008, Chaturvedi et al.
27 92 2014), combining both traits, RWC and membrane dysfunction, would help define physiological
28 93 thresholds for tree mortality.

29 94 The main objective of this study was to identify other physiological traits than the percentage
30 95 loss of hydraulic conductance (PLC) that could work as an indicator of the tree capacity to recover from
31 96 drought. For this, a set of plants of *Prunus lusitanica* L. and *Pseudotsuga menziesii* M., i.e. an
32 97 angiosperm and a conifer species respectively, were exposed to severe drought conditions and allowed
33 98 to dehydrate until the induction of important losses in hydraulic functioning. At this point, trees were
34 99 re-watered to check for the capacity to recover from drought. During the dehydration and the recovery
35 100 phases, we monitored embolism formation and changes in RWC at the stem and the leaf level. We also
36 101 monitored changes in stem diameter to check whether trees were able to recover from drought after
37 102 being re-watered, and assessed the vitality of the stem living tissues. Results also provided us with novel

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3 103 information about the causative relationship between PLC and tree mortality and the level of PLC that
4 104 prevent any recovery from drought in these two species.

7 105 **Materials and methods**

9 106 *Plant material and experimental setup*

11 107 The experiments were carried out in two species: one angiosperm, *Prunus lusitanica* L. and one
12 108 conifer, *Pseudotsuga menziesii* M., a shrub and a tree respectively, selected for their contrasted PLC
13 109 thresholds of drought-induced mortality (i.e. P_{88} and P_{50} respectively). For each species, eight young
14 110 trees were grown under non-limiting water conditions in 5 and 9.2-L pots, respectively, at the INRAE-
15 111 PIAF research station of Clermont-Ferrand, France (45877°N, 3814°E). *P. menziesii* individuals were
16 112 four years old at the time of the experiment while the *P. lusitanica* were two years old. Two weeks
17 113 before starting the experiment, all trees were moved to a controlled-environment glasshouse cell and
18 114 kept under natural light and at a mean temperature of $17.7 \pm 0.2^\circ\text{C}$ (midday) and $10.9 \pm 0.1^\circ\text{C}$ (night).
19 115 During this period, trees were kept well-irrigated (field capacity) by a drip irrigation system controlled
20 116 by an electronic timer. After the two weeks of acclimation, a sub-set of trees for each species (from four
21 117 to six individuals) was exposed to progressive dehydration by withholding the irrigation. In order to
22 118 determine the critical PLC for recovery and because Hammond et al. (2019) reported that conifers were
23 119 able to recover even beyond P_{50} , trees were re-watered to field capacity once reaching water potential
24 120 values corresponding to significant losses in hydraulic functioning according to their vulnerabilities to
25 121 cavitation (i.e. $\text{PLC} > 50\%$ for conifers and $\text{PLC} > 90\%$ for angiosperms). They were then kept well-
26 122 irrigated in order to check for recovery from drought.

27 123 *Vulnerability curves to cavitation*

28 124 Prior to the experiment, the vulnerability to cavitation for the two target species was determined
29 125 to define when trees should be re-watered according to their PLC level. Thus, two different techniques
30 126 (i.e. one technique per species), reported as highly comparable by Brodribb et al. (2017), were used
31 127 according to the xylem characteristics of each species. Thus, for *P. lusitanica*, xylem vulnerability to
32 128 cavitation was determined by using the recently developed optical method (Brodribb et al. 2017) to
33 129 avoid possible biased results related with the open-vessel artefact (Torres-Ruiz et al. 2014, 2015, Choat
34 130 et al. 2016). Indeed, the use of the Cavitron method in this species was not possible due to the length of
35 131 the xylem conduits that were longer than the diameter of the rotor available (Sergent et al. 2020). For *P.*
36 132 *menziesii*, vulnerability curves were constructed by using the Cavitron technique (Cochard 2002) which
37 133 is highly reliable when used to measure species with short conduits such as conifers (Cochard et al.
38 134 2013, Torres-Ruiz et al. 2017). The use of the optical method was not possible for *P. menziesii* because
39 135 the conduits at the stem level are so short that the cavitation events are not always detectable.

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3 136 Briefly, for *P. lusitanica*, the entire plant was let to dehydrate under lab conditions while a clamp
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5 137 equipped with a camera was installed in the stem of four trees after removing the bark carefully with a
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7 138 razor blade to expose undamaged xylem. To avoid the over desiccation of the exposed xylem area during
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9 139 the 6.51 ± 0.52 days of dehydration, we applied a thin coat of silicone grease. The camera then captured
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11 140 images every five min during the dehydration process while changes in stem water potential (Ψ_{stem} , MPa)
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13 141 were continuously monitored using a psychrometer (PSY1, ICT international, Armidale, Australia)
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15 142 installed centrally on the main stem of each plant. The Peltier cooling time was adjusted from 10 s (when
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17 143 the plant was well hydrated) to a maximum of 20 s (as the plant dehydrated) to ensure that sufficient
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19 144 water was condensed onto the thermocouple and then evaporated to produce a stable reading of the wet-
20
21 145 bulb depression temperature. To ensure the accuracy of the measurements obtained with the
22
23 146 psychrometer, regular Ψ_{stem} measurements were carried using a Scholander-type pressure chamber
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25 147 (PMS, Corvallis) in fully developed and healthy leaves previously bagged for at least one hour to prevent
26
27 148 transpiration and promote equilibrium with the plant axis (Fig. S1). Image sequences were then analysed
28
29 149 manually according to Brodribb et al. (2016, 2017). The percentage of embolised pixels for each image
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31 150 was calculated as the amount of embolised pixels cumulated and the total embolised area of the scanned
32
33 151 area. The vulnerability curve was obtained by plotting Ψ_{stem} against cumulative embolisms (% of total).

34
35 152 For *P. menziesii*, xylem vulnerability to cavitation was assessed with the Cavitron technique
36
37 153 (Cochard 2002) which uses centrifugal force to increase the water tension in a xylem segment while
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39 154 measuring the decrease in its hydraulic conductance. Thus, five 0.45m-long stem samples from five
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41 155 different well-hydrated trees (i.e. one sample per tree), were debarked to prevent resin contamination
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43 156 and recut under water with a razor blade to a standard length of 0.27m. For constructing the vulnerability
44
45 157 curves, the maximum sample conductivity (K_{max}) was measured at low speed and relatively high xylem
46
47 158 pressure (-0.75 MPa). The xylem pressure was then decreased stepwise by increasing the rotational
48
49 159 velocity and the conductivity (K) measured at each pressure step. Each pressure was applied on the
50
51 160 sample for two minutes. Sample loss of conductivity (PLC, %) was computed at each pressure as
52
53 161 follows:

$$54 \quad 162 \quad PLC = 100 * \left(1 - \frac{K}{K_{\text{max}}}\right) \quad (1)$$

55
56 163 The resulting curves were fitted according to Pammenter and Vander Willigen equation (1998) and
57
58 164 using the R 'fitPLC' package:

$$59 \quad 165 \quad PLC \text{ or Cumulative embolism} = \frac{100}{(1 + e^{(a/25)(P - P_{50})})} \quad (2)$$

60
61 166 where a is the slope of the curve at the inflexion point, P indicates the xylem water potential for the
62
63 167 optical method (*P. lusitanica*) or the target pressure reached with the Cavitron (*P. menziesii*), and P_{50} is

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3 168 the Ψ_{stem} or pressure value at which 50% of the xylem cavitation events had been observed or at which
4 169 50% loss of conductivity occurred.

6
7 170 *Physiological traits*

9 171 During the progressive dehydration imposed to each subset of plants, Ψ_{stem} was continuously
10 172 assessed by using psychrometers (PSY1, ICT international). Thus, one psychrometer per plant in a total
11 173 of four plants per species was installed at the stem level and covered with aluminium foil to prevent
12 174 their direct exposure to the sunlight and minimize any effect of external temperature variations
13 175 (Vandegheuchte et al. 2014). Psychrometers recorded the Ψ_{stem} every 30 min. To check the accuracy of
14 176 the psychrometers, regular Ψ_{stem} measurements were carried using a Scholander-type pressure chamber
15 177 (PMS, Corvallis) in two fully developed and healthy leaves per plant, previously bagged for at least one
16 178 hour to prevent transpiration and promote equilibrium with the plant axis (Fig. S1).

17 179 Stem diameter variations were monitored continuously by Linear Variable Differential
18 180 Transformer (LVDT) sensors (one LVDT per plant in eight plants per species) installed before
19 181 withholding irrigation. The sensor was applied on the stem with glue and was connected to a data logger
20 182 (Model CR1000, Campbell Scientific LTD) to collect the stem diameter variations (in μm) every 10
21 183 min. By evaluating the dynamics of stem diameter during the dehydration and recovery phases of the
22 184 experiment, we were able to evaluate the capacity of the trees to recover from drought (Lamacque et al.
23 185 2020).

24 186 The RWC was measured at stem (RWC_{Stem}) and leaf level (RWC_{Leaf}) in all trees before
25 187 withholding irrigation (Control) and right before re-watering. RWC_{Stem} and RWC_{Leaf} were calculated
26 188 according to Barrs and Weatherley (1962):

$$27 \text{ 189 } RWC = \frac{(FW - DW)}{(TW - DW)} \quad (3),$$

28 190 where FW is the fresh weight measured immediately after sampling; TW is the turgid weight measured
29 191 after immersing the stem in distilled water for 24 h (for RWC_{Stem}) or after soaking the leaf petiole for 24
30 192 h in distilled water (for RWC_{Leaf}); and DW is the dry weight of the samples after 24 h of drying in an
31 193 oven at 72°C. All measurements were done using a precision scale (METTLER AE 260, DeltaRange
32 194 ®) and were performed on three healthy leaves or one to three small stem sections per plant (depending
33 195 on plant material).

34 196 Once the trees reached water potentials corresponding to a PLC of ca. 88% for *P. lusitanica* and
35 197 50% for *P. menziesii* according to the vulnerability curves, the PLC was assessed in stems using two
36 198 different, but comparable, techniques (Cochard 1992, Torres-Ruiz et al. 2014, Choat et al. 2016). For *P.*
37 199 *lusitanica*, PLC was determined gravimetrically using a xylem embolism meter (XYL'EM, Bronkhorst).
38 200 For *P. menziesii*, as it was impossible to restore the maximal conductance (K_{max}) due to the permanent

201 aspiration of the pit membrane against the cell walls (Cochard et al. 2013), the PLC was assessed by
 202 direct observation using X-Ray microtomography (Micro-CT, Nanotom 180 XS; GE) at the PIAF
 203 laboratory (INRAE) (Cochard et al. 2015). For both techniques, samples were cut progressively
 204 underwater to prevent artefactual increases in the amount of embolism in the samples (Torres-Ruiz et
 205 al. 2015).

206 For the XYL'EM, the PLC was evaluated in three stems (sample length ca. 30mm) per
 207 individual in eight individuals per species. The initial K (K_i) of each segment was determined using a
 208 filtered (0.22 μm) 10 mm KCl and 1 mm CaCl₂ perfusion solution made with distilled water (Cochard
 209 et al. 2009), and applying a pressure head of 8.5 kPa until a steady-state K_i was attained. In order to
 210 determine the maximal conductance (K_{max}), samples from *P. lusitanica* were flushed with water at high
 211 pressure (200 kPa) for 20 minutes to remove all the embolism. PLC was then calculated using the
 212 following equation:

$$213 \quad PLC = 100 * \left(1 - \frac{K_i}{K_{\text{max}}}\right) (4)$$

214 For Micro-CT, one or two samples per plant were collected, as described for the gravimetric K
 215 measurements, and immediately immersed in liquid paraffin wax to prevent dehydration during the
 216 scanning. For each 21-min scan, 1000 images were recorded during the 360° rotation of the sample. The
 217 X-ray setup was fixed at 70kV and 240 μA . At the end of the experiment, samples were cut 3mm above
 218 the scanned cross-section, injected with air (0.1MPa) and re-scanned to visualize all the conduits filled
 219 with air. The amount of PLC was computed by determining the ratio between the amount of cavitated
 220 conduits in the samples before and after cutting the sample.

221 *Cell vitality*

222 Cell vitality was assessed using two different methods: the electrolytes leakage test (EL) (Zhang
 223 and Willison 1987, Sutinen et al. 1992) and a fluorescein diacetate (FDA) staining process (Widholm
 224 1972). Cell vitality was assessed in both control and drying trees right before rewatering the latter ones.
 225 For EL, one to three stem samples per plant (depending on plant material availability) were cut into ten
 226 2-mm thick slices and immersed in test tubes containing 15mL of pure water. Test tubes were shaken at
 227 60 shakes per min during 24h at 5°C to stop enzyme activity. Water conductivity of the effusate (C1)
 228 was then measured at room temperature using a conductimeter (3310 SET1, Tetracon® 325, WTW,
 229 Weilheim, Germany). Then, all the living cells were killed by autoclaving the samples at 121°C for 30
 230 min (King and Ludford 1983), cooled down at room temperature (22°C approx.) for 60 min and the
 231 effusate maximal conductivity (C2) measured. The lysis percentage (EL) was then determined as:

$$232 \quad EL = \frac{C1}{C2} * 100 (5)$$

233 To stain the cytoplasm of stem living cells and quantify the amount of living cells and their
 234 location for each individual, FDA (F7378-10G, SIGMA-ALDRICH) was used. For this, two or three 60
 235 μm -thick stem cross-sections were obtained with a microtome (Leica RM2165) and stained for 20 min
 236 in a 1% FDA solution (Widholm 1972). Cross-sections were observed using an inverted fluorescence
 237 microscope (Axio Observer Z1, ZEISS; Bright light or YFP filter) within the next hour after staining.
 238 An entire cross-section image was obtained by joining images with the same magnification taken from
 239 all the cross-section of the sample for both bright light and fluorescence observations. The percentage
 240 of bark living cells (BLC) for each cross-section was calculated as follow:

$$BLC = \frac{FA}{BA} * 100 \quad (6)$$

242 Where FA is the total fluorescent area of the sample and BA is the bark area determined using Fiji
 243 software (Schindelin et al. 2012).

244 *Statistical analyses*

245 Statistical analyses consisted of paired *t*-test (after testing for normality and homogeneity of
 246 variances) and Wilcoxon test (for non-normal distribution) and were performed using R programs to
 247 compare the set before the drought event (Control) and before re-watering. All tests were performed
 248 using a level of significance $\alpha = 0.05$.

249 **Results**

250 *Capacity of recovery from drought*

251 Vulnerability curves reported P_{50} -values of -6.07 and -3.73MPa for *P. lusitanica* and *P.*
 252 *menziesii*, respectively (Fig. 1). *P. lusitanica* individuals were thus rehydrated once they reached water
 253 potential values of ca. -9.0 to -10.0MPa i.e. above its P_{88} of -8.94MPa. *P. menziesii* were rehydrated
 254 once showing water potential of ca. -7.0 to -10.0 MPa ($P_{88} = -5.34\text{MPa}$).

255 In control conditions, the mean levels of PLC in the stem for *P. lusitanica* and *P. menziesii* were
 256 6.9 (± 3.5 SE) and 7.40 (± 2.8 SE) respectively (Fig. 2). Right before applying the recovery irrigation, the
 257 mean PLC for *P. lusitanica* and *P. menziesii* were 94.4 (± 1.98 SE) and 79.5 (± 3.7 SE) respectively
 258 (Table S1) i.e. above the current point for xylem hydraulic failure for angiosperms (i.e. P_{88}) and conifers
 259 (i.e. P_{50}).

260 Stems showed a noticeable shrinkage for both species during the time-course of the dehydration
 261 for all individuals (Fig. 3). After rewatering, two *P. lusitanica* individuals that reached a mean PLC of
 262 90.3 (± 8.3 SE) (Fig. 2A) showed an increase in stem diameter immediately after being re-hydrated and
 263 were considered as recovered trees (Fig. 3A). On the contrary, the six individuals that reached PLC of
 264 95.8 (± 1.1 SE) showed a continuous decrease in stem diameter after the rehydration and were considered

265 as dead trees (Fig. 3C). For *P. menziesii*, only one individual that reached a Ψ_{stem} value of -7.48MPa and
 266 a PLC level of 67.9 (Fig. 2B) was able to recover in terms of trunk diameter after rewatering (Fig. 3B).
 267 All the other individuals continued to show a decrease in stem diameter during the re-watering phase
 268 after reaching a mean Ψ_{stem} value of -8.7MPa (± 0.5 SE) (Fig. 3D) and a mean PLC of 81.1 (± 3.8 SE)
 269 (Fig. 2B).

270 For both species, individuals that were able to recover from drought showed an increase in Ψ_{stem}
 271 concomitantly to the increase in stem diameter (Fig. 3A, B) while no recovery in Ψ_{stem} was noticed in
 272 trees considered as dead (Fig. 3C and Fig. 3D).

273 A significant decrease in RWC_{Stem} was observed for both species during dehydration as PLC
 274 increases (Fig. 4A and Fig. 4B; Table S2). In control *P. lusitanica* trees, RWC_{Stem} was of 92.3% (± 0.8
 275 SE) whereas it dropped for those exposed to drought to 58.5% (± 1.5 SE) for recovered individuals and
 276 to 54.7% (± 3.6 SE) for dead individuals before re-watering. Differences in RWC_{Stem} , however, were not
 277 significant when comparing recovered and dead individuals. Similar results were observed for *P.*
 278 *menziesii*, with a significant decrease in RWC_{Stem} noticed for both recovered and dead individuals from
 279 drought. Thus, RWC_{Stem} decreased from 83.4% (± 1.1 SE) for control trees to 49.8% for recovered and
 280 36.9% (± 1.9 SE) for dead trees.

281 Similar to RWC_{Stem} , RWC_{Leaf} was significantly impacted in both species during dehydration
 282 (Fig. 4C, D; Table S2). For *P. lusitanica*, RWC_{Leaf} decreased from 94.8 % (± 0.5 SE) (Control) to 56.9%
 283 (± 4.1 SE) in plants that recovered from drought and to 59.3 % (± 4.8 SE) in those that did not recover.
 284 For *P. menziesii*, RWC_{Leaf} went from 92.4 % (± 2.0 SE) (Control) to 53.5 % in recovered trees or 51.5
 285 % (± 4.7 SE) in dead trees. Differences in RWC_{Leaf} were not significant for any of the two species when
 286 comparing recovered and dead individuals.

287 *Tissue vitality*

288 For *P. lusitanica*, all trees showed higher EL values than control ones before re-watering (Fig.
 289 4E and Fig. 4F, Table S2) (Control: 29.9% ± 1.3 SE; Recovered: 47.12% ± 7.12 SE; Dead: 57.2% ± 6.7
 290 SE). However, no differences were noticed when comparing recovered and dead individuals before re-
 291 watering (Recovered: 47.1% ± 7.1 SE; Dead: 57.2% ± 6.7 SE). For, *P. menziesii*, only the trees that did
 292 not recover showed higher EL values compared to control (Control: 50.6% ± 2.2 SE; Dead: 78.8% ± 2.2
 293 SE). No differences in EL were observed between the recovered individual and control ones (Control:
 294 50.6% ± 2.2 SE; Recovered: 50.8%). The recovered individual tends to show lower EL values than the
 295 dead ones (Recovered: 50.8%; Dead: 78.8% ± 1.2 SE).

296 In control trees and for both species, the FDA staining showed that living cells were mostly
 297 located at the outer bark and phloem level (Fig. 5). Before re-watering, the amount of living cells in *P.*
 298 *lusitanica* decreased noticeably in dead trees (Fig. 6A; Table S3) (Control: 23.0% ± 4.4 SE; Dead: 3.0%

299 ± 1.4 SE) but not in trees that recovered (Control: 23.0% ± 2.4 SE; Recovered: 15.3% ± 10.4 SE). For *P.*
300 *menziesii*, the amount of living cells decreased in trees that did not recover (Control: 10.2% ± 2.1 SE;
301 Dead: 0.8% ± 0.6 SE) while no noticeable decrease was encountered in trees that recovered (Control:
302 10.2% ± 2.2 SE; R: 7.2%) (Fig. 6B; Table S3).

303 Discussion

304 Our results provide strong evidence that even when presenting high levels of hydraulic
305 dysfunction, trees were able to recover from an extreme drought event after being re-watered. Indeed,
306 *P. lusitanica* individuals that showed PLC values of 98.6%, i.e. well above the suggested threshold for
307 recovery and point of death for angiosperms (P_{88} , Barigah et al. 2013; Urli et al. 2013), recovered from
308 drought according to their stem diameter dynamic (i.e. showed an increase in stem diameter immediately
309 after re-watering) and even flushed new leaves after being re-watered at field capacity (Fig. S2).
310 Similarly, *P. menziesii* individuals with PLC values of 67.9%, i.e. above the threshold for recovery for
311 conifers (P_{50} , Brodribb and Cochard 2009), were also able to recover once re-watered. These results,
312 therefore, demonstrate how trees are able to recover from drought even when their PLC levels reach
313 higher values than those considered as threshold for recovery for angiosperms and conifers (i.e. P_{88} and
314 P_{50} , respectively). These results agree with those provided for loblolly pine (*Pinus taeda* L.) by
315 Hammond et al. (2019) which reported a higher chance for trees to die than to survive once reaching
316 PLC levels of 80, i.e. much higher than the P_{50} threshold commonly reported for conifers. In our study,
317 however, no recovery was observed for *P. menziesii* when PLC reached values above 68%, which raises
318 questions on how lethal PLC thresholds vary among tree species. For angiosperms, our results also agree
319 with the ones provided for *Pistacia lentiscus* L. by Vilagrosa et al. (2003) that show how drought-
320 induced mortality only occur in plants that reached PLC values of almost 100%. When taken together,
321 all these results highlight the importance of revising the actual recovery and point of death thresholds
322 suggested for angiosperms and conifers. More importantly, these results show that plant mortality occurs
323 when the losses in xylem conductance are important (e.g. >90% of xylem hydraulic dysfunction),
324 suggesting that PLC is not the sole triggering mechanism of plant death under drought conditions.

325 Unfortunately, any critical thresholds for most of the physiological traits monitored during our
326 experiment were identified as a potential proxy for drought-induced mortality. Thus, it was not possible
327 to evince a clear causal link between stem hydraulic failure and plant mortality since trees that were not
328 able to recover from drought did not consistently show higher PLC values than those that survived.
329 However, two interesting trends emerged from the RWC_{Stem} and EL results for *P. menziesii*. On one
330 hand, trees recovering from drought tend to show higher RWC_{Stem} than dead ones before re-watering
331 and, on the other hand, trees that recovered tend to show lower EL values than the dead ones. These
332 results agree with Martinez-Vilalta et al. (2019) and highlight the importance of plant water content as
333 a potential indicator of mortality risk. However, this was not the case for *P. lusitanica* since similar

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3 334 RWC_{Stem} values were observed in both trees that were able to recover from drought and those that were
4 335 not. This raises the possibility of using RWC_{Stem} as a proxy for mortality across species, although more
5 336 confirmatory studies should be carried out especially in angiosperms. At leaf level, RWC_{Leaf} at turgor
6 337 loss point is relatively high and constant between species (Bartlett et al. 2012), which potentially could
7 338 make it a useful trait for identifying survival events since noticeable changes in RWC_{Leaf} would occur
8 339 at high dehydration level preceding death (Martinez-Vilalta et al. 2019). However, no differences in
9 340 RWC_{Leaf} were detected in our study between recovering and dead trees before re-watering for any of the
10 341 two species evaluated probably because, at those levels of water stress, leaves were already hydraulically
11 342 disconnected from the stems in all the individuals. This would favour a faster dehydration of the leaves
12 343 in comparison with the stems and, therefore, may partially explain the similarly low values for RWC_{Leaf} .
13 344 Therefore, rather than just focusing only on the plant water status, a deeper study on water relocation in
14 345 trees during drought (Körner 2019) would be required for identifying potential proxies for drought-
15 346 induced mortality. In fact, a crucial question now is to evaluate if the relocation of water from plant
16 347 reserves would be enough for keeping key tree tissues hydrated during drought and, therefore, enhancing
17 348 plant probability of survival after re-watering (Holbrook 1995).

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19 349 Regarding cell integrity, recovering trees from drought tend to present lower cell damages than
20 350 the dead trees before re-watering. *P. menziesii* recovering trees showed seemingly no changes in their
21 351 percentage of EL even after the drought event. Dead trees, on the contrary, consistently showed higher
22 352 EL values before re-watering than control trees able to recover in agreement with the results reported
23 353 by Vilagrosa et al. (2010) for *P. lentiscus*. Even if *P. lusitanica* dead and recovering trees did not show
24 354 any differences in EL, recovering trees were able to resprout and flush new leaves when the stress was
25 355 alleviated (Fig. S2). As higher EL values are the consequences of membrane failure and are associated
26 356 with cell death (Vilagrosa et al. 2010, Guadagno et al. 2017), these observations suggest that the fatal
27 357 failure at the cellular scale does not occur homogeneously within the stem and this, as shown by Thomas
28 358 (2013) and Klimešová et al. (2015), allow the resprouting of the plant if the stress is relieved. Therefore,
29 359 according to our results, the membrane integrity could emerge as a proxy for lack of recovery capacity
30 360 in conifers since the cell vitality in some of the living tissues at the stem level seems to have a relevant
31 361 role in drought-induced mortality. However, the link between membrane failure and the loss in stem
32 362 hydraulic functioning is still unresolved. Indeed, it is still unclear whether the extreme dehydration leads
33 363 to membrane failure through physical (i.e. cell cavitation, Sakes et al. 2016), collapse and cytorrhysis
34 364 (Taiz and Zeiger 2006) or only biochemical processes (Suzuki et al. 2012, Wang et al. 2013, Petrov et
35 365 al. 2015).

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37 366 The presence of living cells in stems at the inner bark level was not always related to the survival
38 367 of the trees after re-watering (i.e. increase in stem diameter). This was the case for *P. lusitanica* where
39 368 trees showing similar amounts of living cells, differed in their capacity to recover from drought. The
40 369 presence of living cells in dead trees could be explained by the fact that, under drought conditions, trees

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3 370 can rely on their own water reserves (Epila et al. 2017) which could temporally maintain the metabolism
4 371 of the cell despite being hydraulically disconnected from the roots. However, once the water reserves
5 372 are depleted, living tissues would ultimately dry and cells would dehydrate and die. Therefore, not only
6 373 the presence of living cells is required for allowing the plant to recover from drought but also their
7 374 hydraulic connection with the other plant tissues and organs upstream. Thus, even at stem PLC values
8 375 near to 100% for angiosperms or well above 50% for conifers, a minimal hydraulic connection between
9 376 the soil and the living tissues could be enough to recover from drought if plants have access to water.
10 377 More studies focused on the link between xylem hydraulic functioning, plant capacitance and cell
11 378 mortality are therefore required to identify what the thresholds for tree survival to drought are.

17 18 379 **Conclusion**

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20 380 By combining a living-cell staining process with LVDT sensors and PLC measurements, this
21 381 study showed that the common thresholds for recovery and point of death considered until now, i.e P_{50}
22 382 for conifers and P_{88} for angiosperms, are not accurate enough for assessing and predicting drought-
23 383 induced tree mortality. Indeed, our results showed that trees with PLC levels of 98.6% for *P. lusitanica*
24 384 (angiosperm) and 67.9% for *P. menziesii* (conifer) were still able to recover from drought once re-
25 385 watered. Thus, even if the link between a high level of stem PLC and tree mortality is clear, there is an
26 386 urgent need in defining new physiological thresholds for predicting tree mortality with mechanistic
27 387 models. For conifers, higher RWC_{Stem} and lower EL values were related to a higher capacity to survive
28 388 drought. However, this was not the case for angiosperms for which no physiological traits were
29 389 identified as a potential proxy for the capacity of plant to recover although a similar pattern as to the one
30 390 observed for the conifer species was identified.

31 32 33 34 35 36 37 38 391 **Author contributions**

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40 392 MM and JMTR conceived and designed the experiment. MM and PEMS were responsible for
41 393 running the measurements and carried out the data analysis. EB supervised the setting up of the micro-
42 394 CT scans. MM, PEMS, HC and JMTR interpreted the results. MM wrote the first manuscript draft.
43 395 JMTR, PEMS, HC and EB assisted substantially with manuscript development.

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48
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52 53 54 55 400 **Data availability statement**

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57 401 The data are not publicly available due to privacy restrictions.
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For Peer Review

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591 **Supporting information**

592 Additional supporting information may be found online in the Supporting Information section at the end
593 of the article:

594 **Table S1.** PLC evolution during the time-course of the experiment.

595 **Table S2.** Evolution of the stem relative water content (RWC_{Stem}), leaf relative water content (RWC_{Leaf})
 596 and electrolytes leakage (EL) during the time-course of the experiment

597 **Table S3.** Evolution of the percentage of bark living cells (%BLC) during the time-course of the
 598 experiment.

599 **Figure S1.** Validation of the Ψ_{stem} measurements recorded with psychrometer and compared to the Ψ_{stem}
 600 measurements carried out with the Scholander pressure chamber on previously bagged leaves.

601 **Figure S2.** Plants flushing new leaves after re-watering.

602 **Figures legends**

603 Figure 1. Vulnerability curves to cavitation for *P. lusitanica* stems and *P. menziesii* stems. Vulnerability
 604 curve for *P. lusitanica* stems obtained on four different samples using the optical method (Brodrribb et
 605 al. 2017). The P_{50} is evaluated at -6.07MPa while the P_{88} is evaluated at -8.94MPa. Vulnerability curve
 606 for *P. menziesii* stems obtained on five different samples using the Cavitrone technique developed by
 607 Cochard in 2002. The P_{50} is evaluated at -3.73MPa and P_{88} is evaluated at -5.34MPa. Red solid lines
 608 represent the P_{50} while red dashed lines represent the confidence interval around P_{50} at 95%. Violet and
 609 green rectangles correspond to the water potential values at which *P. lusitanica* and *P. menziesii* were
 610 respectively irrigated.

611 Figure 2. Box plots representing the dispersions of percentage loss of conductance (PLC) values for A
 612 *P. lusitanica* and B *P. menziesii* before water stress (control) and before re-watering for recovering (R)
 613 and dead (D) trees measured with the Xyl'EM apparatus for *P. lusitanica* and X-ray micro-CT for *P.*
 614 *menziesii*.

615 Figure 3. Dynamic of the stem diameter (solid line) and evolution of the water potential (points) during
 616 the time-course of the experiment. Stem diameter dynamic (in μm) was recorded by Linear Variable
 617 Differential Transformer (LVDT) for both species while the water potential was measured punctually
 618 using a Scholander pressure chamber for *P. lusitanica* individuals and continuously by psychrometers
 619 for *P. menziesii* individuals. The light grey rectangles represent the period where water was withheld to
 620 simulate an extreme drought event. The red line indicates the percentage loss of conductance (PLC)
 621 value at which the plant was re-watered. Panels A and B show the recovery of individuals after re-
 622 watering in terms of stem diameter while panels C and D show dead individuals.

623 Figure 4. Variation of Stem Relative Water Content (RWC_{Stem}) (panels A and B), Leaf Relative Water
 624 Content (RWC_{Leaf}) (panels C and D), stem Electrolyte Leakage (EL) (panels E and F) for *P. lusitanica*
 625 and *P. menziesii*. Measurements were performed on all individuals in control conditions (Control) and
 626 after the drought event (i.e. before the rehydration of the plants for recovered and dead individuals).

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3 627 Figure 5. Cross-sections of *P. lusitanica* (A and B) and *P. menziesii* (C and D) stems in control
4 628 conditions. Cross-sections were stained using fluorescein diacetate (FDA) (60 μ m thick cross-section –
5 629 1% solution) and microphotographs were taken using a bright light (A and C) and an inverted
6 630 fluorescence microscope (YFP filter; B and D). Living cells (fluorescent spots) are located in the phloem
7 631 and outer bark for both species.

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11 632 Figure 6. Percentage of bark living cells (%BLC) stained with FDA in stem cross-sections in *P.*
12 633 *lusitanica* (panel A) and *P. menziesii* (panel B). “R” refers to recovering trees and “D” refers to dead
13 634 trees.

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15
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17 635 Table S1. Table summarizing the evolution of the PLC during the time-course of the experiment in A
18 636 *P. lusitanica* and B *P. menziesii*. Control values represent the mean value of the measurements
19 637 performed before the drought event. BRW represents the measurements performed on the individuals
20 638 the day of the rehydration.

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22
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24 639 Table S2. Table summarizing the evolution of the stem relative water content (RWC_{Stem}), leaf relative
25 640 water content (RWC_{Leaf}) and electrolytes leakage (EL) during the time-course of the experiment in A *P.*
26 641 *lusitanica* and B *P. menziesii*. Control values represent the mean value of the measurements performed
27 642 before the drought event. BRW represents the measurements performed on the individuals the day of
28 643 the rehydration.

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32 644 Table S3. Table summarizing the evolution of the percentage of bark living cells (%BLC) during the
33 645 time-course of the experiment in A *P. lusitanica* and B *P. menziesii*. Control values represent the mean
34 646 value of the measurements performed before the drought event. BRW represents the measurements
35 647 performed on the individuals the day of the rehydration.

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39 648 Figure S1. Validation of the stem water potential (Ψ_{stem}) measurements recorded with psychrometer and
40 649 compared to the Ψ_{stem} measurements carried out with the Scholander pressure chamber on previously
41 650 bagged leaves. A for *P. lusitanica* and B for *P. menziesii*.

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45 651 Figure S2. Photographs of *P. lusitanica* plants flushing new leaves after experimenting a drought event
46 652 and reaching levels of PLC of 98.6%. A 19 days after re-watering; B 28 days after re-watering.

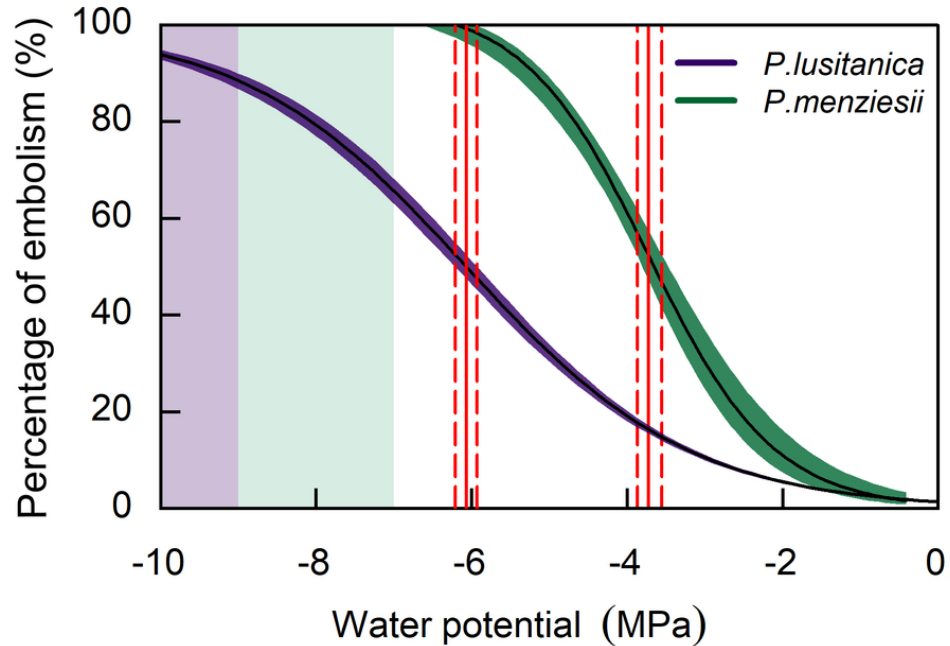
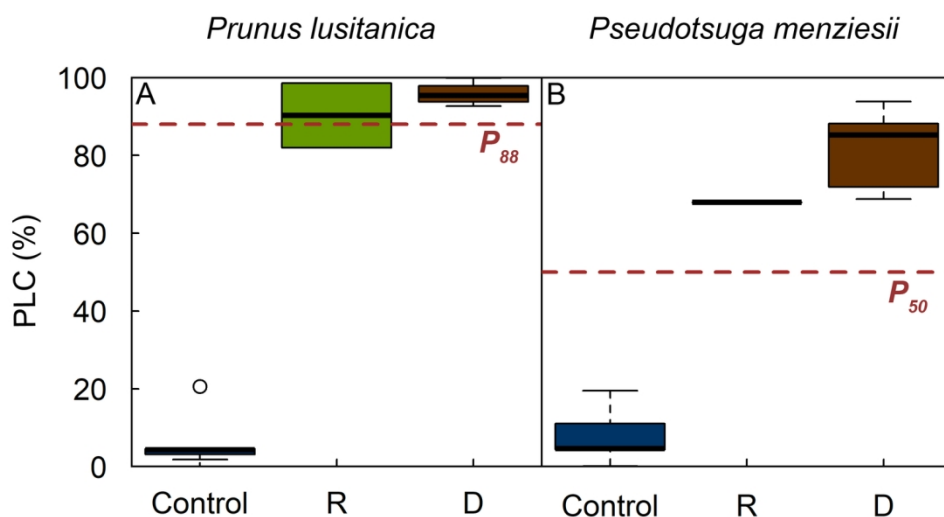


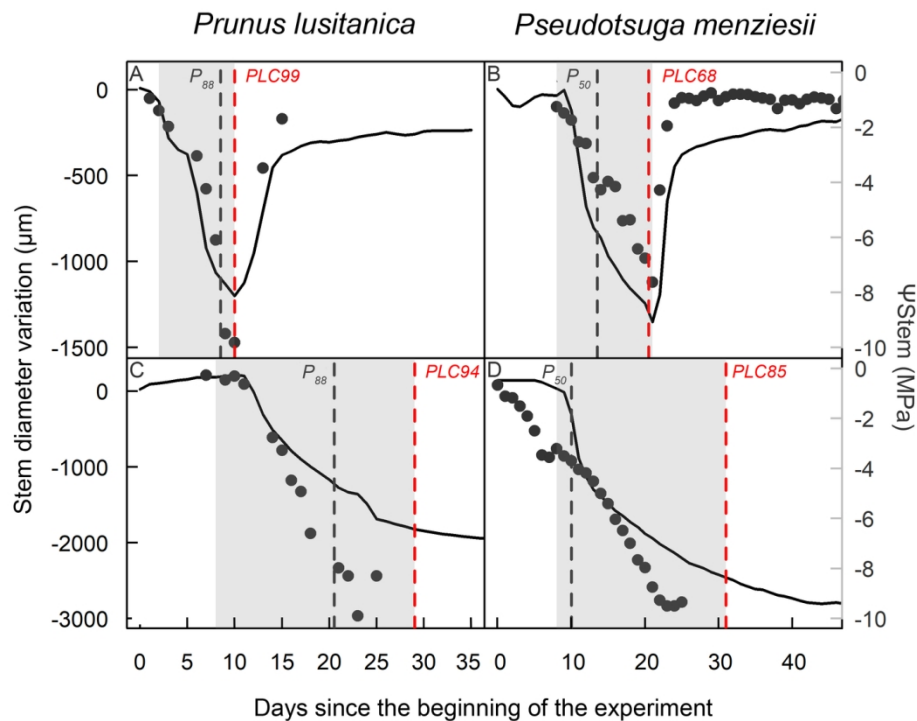
Figure 1. Vulnerability curves to cavitation for *Prunus lusitanica* L stems and *Pseudotsuga menziesii* M stems. Vulnerability curve for *P. lusitanica* stems obtained on four different samples using the optical method (Brodribb *et al.* 2017). The P_{50} is evaluated at -6.07MPa while the P_{88} is evaluated at -8.94MPa. Vulnerability curve for *P. menziesii* stems obtained on five different samples using the Cavitron technique developed by Cochard in 2002. The P_{50} is evaluated at -3.73MPa and P_{88} is evaluated at -5.34MPa. Red solid lines represent the P_{50} while red dashed lines represent the confidence interval around P_{50} at 95%. Violet and green rectangle correspond to the water potential values at which *P. lusitanica* and *P. menziesii* were respectively irrigated.

79x59mm (300 x 300 DPI)



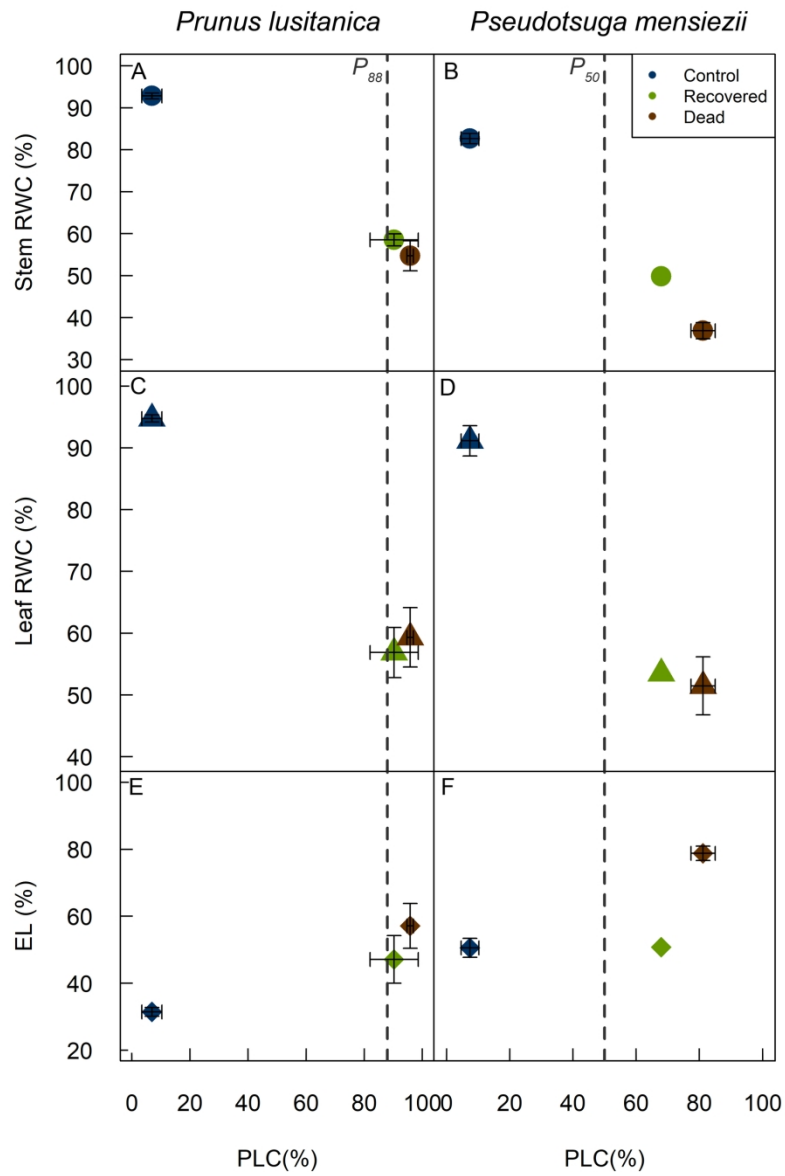
Box plots represents the dispersions of percentage loss of conductance (PLC) values for **A** *Prunus lusitanica*, **L** and **B** *Pseudotsuga menziesii*. M before water stress (control) and before re-watering for recovering (R) and dead (D) trees measured with the Xyl'em apparatus for *P. lusitanica* and X-ray micro-CT for *P. menziesii*.

140x79mm (300 x 300 DPI)



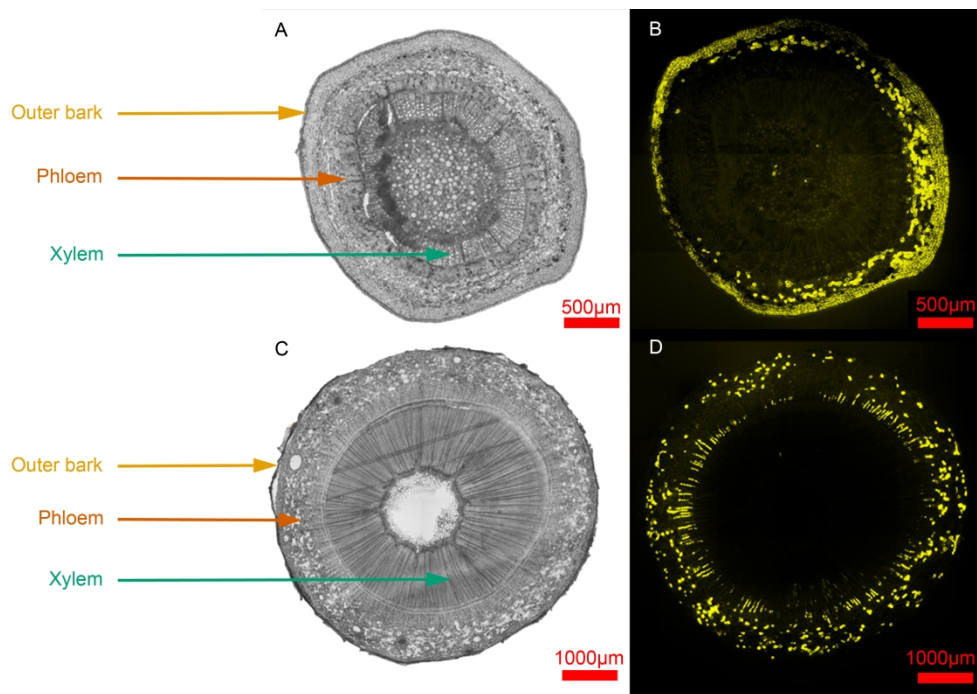
Dynamic of the stem diameter (solid line) and evolution of the water potential (points) during the time-course of the experiment. Stem diameter dynamic (in μm) was recorded by LVDT for both species while the water potential was measured punctually using a Scholander pressure chamber for *Prunus lusitanica* individuals and continuously by psychrometers for *Pseudotsuga menziesii* individuals. The light grey rectangles represent the period where water was withheld to simulate a extreme drought event. Panels **A** and **B** show the recovery of individuals after re-watering in terms of stem diameter while panels **C** and **D** show dead individuals.

132x99mm (300 x 300 DPI)

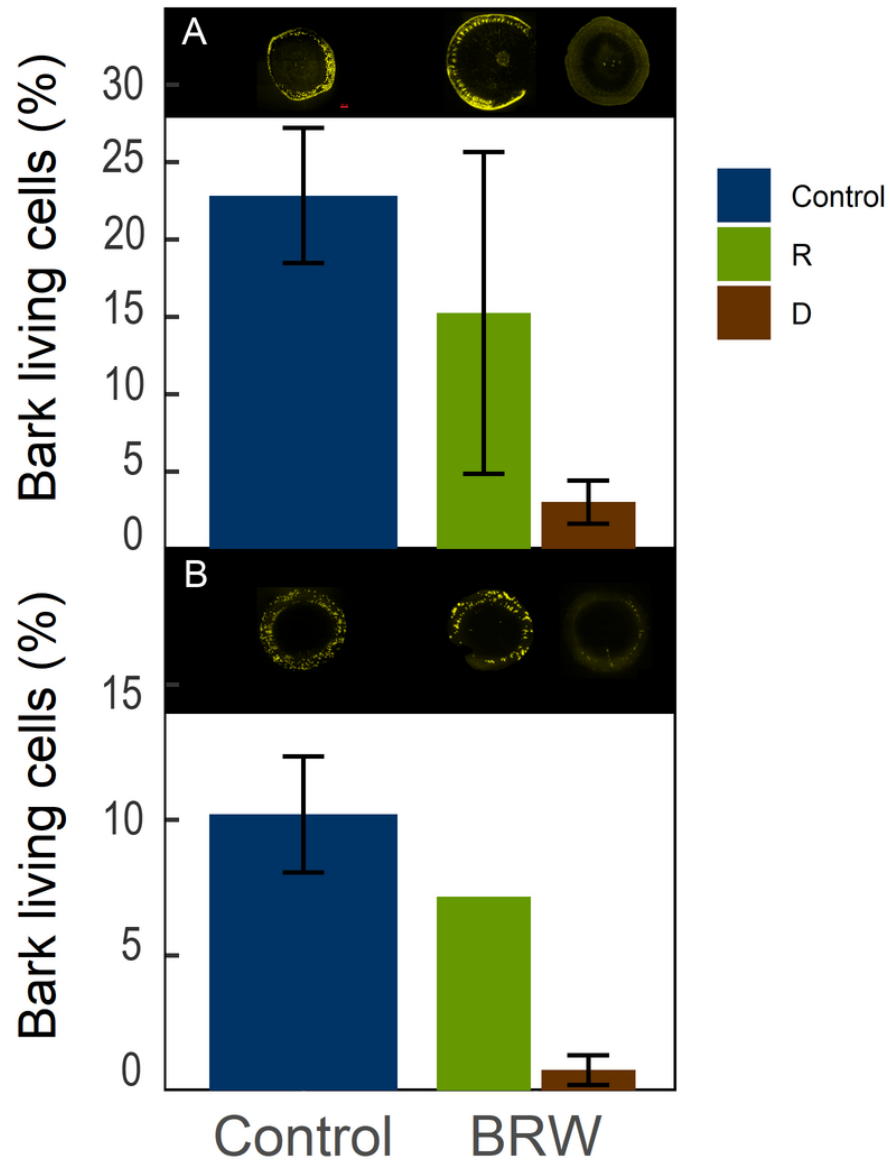


Variation of Stem Relative Water Content (RWC_{Stem}) (panels **A** and **B**), Leaf Relative Water Content (RWC_{Leaf}) (panels **C** and **D**), stem Electrolyte Leakage (EL) (panels **E** and **F**) for *Prunus lusitanica* L and *Pseudotsuga menziesii* M. Measurements were performed on all individuals in control conditions (Control) and after the drought event (e.g. before the rehydration of the plants for recovered and dead individuals).

165x219mm (300 x 300 DPI)



Cross sections of *Prunus lusitanica* L (**A** and **B**) and *Pseudotsuga menziesii* M (**C** and **D**) stems in control conditions. Cross sections were stained using fluorescein diacetate (FDA) (60µm thick cross section – 1% solution) and microphotographs were taken using a bright light (**A** and **C**) and an inverted fluorescence microscope (YFP filter **B** and **D**). Living cells (fluorescent spots) are located in the phloem and outer bark for both species.



Percentage of bark living cells (%BLC) stained with FDA in stem cross section in *Prunus lusitanica* L (panel **A**) and *Pseudotsuga menziesii* M (panel **B**). "R" refers to recovering trees and "D" refers to dead trees.

68x90mm (300 x 300 DPI)