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## Hydraulic failure and tree mortality: from correlation to causation

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1 **TITLE**

2 Hydraulic failure and tree mortality: From correlation to causation

3 **AUTHOR NAMES**

4 Marylou Mantova, Stéphane Herbette, Hervé Cochard, José M. Torres-Ruiz.

5 **HIGHLIGHTS**

- 6
- Xylem hydraulic failure has been shown to be a ubiquitous factor for tree death from drought, but the mechanistic link between the two processes remains unclear.
  - As meristematic cells are involved in the recovery of trees from drought, determining the damage they suffer during their progressive dehydration under water stress and its relationship to the loss of hydraulic function will provide new information on the mechanistic link between hydraulic failure and drought-induced mortality.
  - The RWC, altered redox status and physical constraints meristematic cells can support more accurately define meristem membrane integrity, and consequently meristems survival and so tree recovery, than the  $P_{50}$  and  $P_{88}$  values used hitherto as thresholds for drought-induced tree mortality in mechanistic models.
  - The capacity of trees to relocate stored water between tissues is crucial for buffering the variation in the RWC and redox status of meristematic cells, determining the cell mortality timeline and therefore the resistance of different species to drought.
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19

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5 **OUTSTANDING QUESTIONS**

- 6
- What is the sequence of events ending in meristematic cells death?
- 7
- Is the hydraulic failure of the tree water transport system the triggering factor in the
- 8
- irreversible dehydration of meristematic cells?
- 9
- What level of hydraulic dysfunction is necessary to cause a reduction in RWC that
- 10
- induces significant damage to meristematic tissues?
- 11
- Does this hydraulic dysfunction level vary among species with different drought
- 12
- resistance strategies?
- 13
- Are ROS accumulation and RWC level critical for meristematic cells survival?
- 14
- How do critical ROS and RWC vary with drought intensity and among species?
- 15
- What level of cell damage can trees withstand before they lose their ability to recover,
- 16
- and so die from drought?
- 17
- Can meristematic cells dehydration be delayed through radial relocation of water in the
- 18
- stem during drought?

[Click here to view linked References](#)

1 **Opinion article**

2 **TITLE**

3 Hydraulic failure and tree mortality: From correlation to causation

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18 **KEYWORDS**

19 Tree mortality, meristems mortality, hydraulic functioning, membrane failure, water content

20

21 **GLOSSARY**

22 **Apoplastic water:** Water stored outside cell plasmalemma: e.g. cell wall, intercellular spaces,  
23 or in dead cells: e.g. vessels, fibres, tracheids.

24 **Cellular death:** A cell is considered dead when: (i) the cell has lost plasma membrane integrity;  
25 (ii) the cell, including its nucleus, has undergone complete fragmentation into apoptotic bodies;  
26 or (iii) cell fragments have been engulfed by an adjacent cell *in vivo*.

27 **Cellular cavitation:** Cellular cavitation occurs when the evaporation of water from the cell  
28 causes the radial walls to come closer and the lateral wall to cave inwards. When a critical  
29 pressure is reached, the cytoplasm fractures and a gas bubble forms inside the cell.

30 **Cytorrhysis:** The permanent and irreparable shrinkage of the cell resulting in the cell wall  
31 being mechanically deformed as the cell loses volume due to the loss of internal positive  
32 pressure.

33 **Hydraulic failure:** A physiological status in which the loss of hydraulic conductance of the  
34 xylem undergoes a runaway effect leading to the irreversible dehydration of the distal organs.

35 **Necrotic plant cell death or necrosis:** Cell injury leading to premature cell death typically  
36 observed in cells undergoing abiotic stress. Necrosis is an acute cell death response that  
37 develops rapidly in several minutes to one day.

38 **Primary meristems:** Type of meristematic tissues responsible for the primary growth of the  
39 plant (e.g. apical meristems), i.e. growth in height or length. Primary meristems are directly  
40 derived from embryonic cells.

41 **ROS:** Reactive oxygen species. Oxygenated chemical species such as free radicals, oxygen  
42 ions and peroxides that are made chemically highly reactive by the presence of unpaired valence  
43 electrons.

44 **RWC:** Relative water content. Indicator of the water status of a tree organ or tissue. Calculated  
45 relative to the water status at the saturation.

46 **Secondary meristems:** Type of meristematic tissues responsible for the secondary growth of  
47 the plant (e.g. cambium, phellem), i.e. growth in girth or thickness. Secondary meristems are  
48 derived from the permanent tissues.

49 **Symplastic water:** Refers to the water stored in the cell plasmalemma, corresponding to the  
50 intracellular continuum formed by plant cells through plasmodesmata.

51 **Tree death/mortality:** Irreversible cessation of the metabolism in a tree. From a plant-water  
52 relation point of view, tree death occurs when a tree is no longer able to maintain its key  
53 physiological functions, e.g. growth and or reproduction.

54

## 55 **ABSTRACT**

56 Xylem hydraulic failure has been recognized as a pervasive factor for triggering drought-  
57 induced tree mortality. However, foundational evidence of the mechanistic link connecting  
58 hydraulic failure with living cell damages and tree death has not been identified yet,  
59 compromising our ability to predict mortality events. Meristematic cells are involved in the  
60 recovery of trees from drought and focusing on their vitality and functionality after a drought  
61 event could provide novel information on the mechanistic link between hydraulic failure and  
62 drought-induced tree mortality. This opinion piece focuses on the cell critical hydration status  
63 for tree recovering from drought and how it links with the membrane integrity of the meristems.

64

## 65 **MAIN TEXT**

### 66 **Dying of thirst**

67 Ongoing climate change is modifying surface temperature and precipitation patterns in  
68 many areas worldwide [1]. As a consequence, drought episodes are more frequent, longer, and  
69 more intense [2], having already a marked impact on tree survival and forest dieback [3,4].  
70 There is therefore an urgent need to predict what species will succumb to drought and where  
71 and when this will occur [5] in order to anticipate the degree to which the expected future  
72 climatic changes will affect forests structure and function.

73 Physiologically, **tree death** (see Glossary) is usually assessed by evaluating a tree's  
74 ability to recover its key physiological functions, such as exchanges of matter and energy with  
75 its environment, or resprout, that is to say produce or regenerate new organs or tissues in the  
76 following vegetative season [3,6,7]. A tree is considered to have died as a result of a drought  
77 episode when, once exposed to more favourable conditions, it is unable to perform these key  
78 physiological functions. The ability of a tree to survive a drought episode is therefore linked to

79 its ability to ensure the survival of the key meristematic cells, both **primary** and **secondary**,  
80 such as undifferentiated meristematic apical cells responsible for the development of new  
81 organs, cambium cells involved in the development of vascular tissues and the subero-  
82 phelloderm cells responsible for the development of bark tissues, as well as root and shoot  
83 apical meristems responsible for the primary growth of the plant. If a drought episode is intense  
84 enough to affect the vitality of one or more of these key meristematic tissues, it may hinder the  
85 tree's ability to maintain its metabolism and ultimately to survive.

86 In the last decades, much effort has been made to understand tree hydraulic functioning  
87 and especially the link between **xylem embolism** and drought-induced tree mortality [8].  
88 Embolism occurs when excessive tensions in the xylem tissue cause cavitation events in the  
89 xylem conduits that block water transport from the roots to the leaves. Cavitation occurs under  
90 severe drought conditions and has been identified as a ubiquitous factor for tree death from  
91 drought (Box 1) [9]. Several studies have shown that the water potential inducing a loss of  
92 hydraulic functioning of ca. 50% or ca. 88% ( $P_{50}$  for conifers and  $P_{88}$  for angiosperms,  
93 respectively) [6,7], can be considered as an indicator for drought-induced tree mortality or lack  
94 of full recovery in some species [10–12]. These threshold levels of embolism thus define the  
95 point of **hydraulic failure** of the xylem tissue that correspond to the point where the  
96 conductance capacity of the sap pathway is unable to avert the runaway cavitation of the xylem  
97 leading to the progressive dehydration of the distal organs. The process of cavitation has been  
98 widely studied, and the critical  $P_{50}$  and  $P_{88}$  values have been determined for different species  
99 [13,14] and used as thresholds for modelling tree mortality under drought conditions [15,16].  
100 However, although  $P_{50}$  and  $P_{88}$  have been strongly correlated to tree resistance to drought in  
101 many cases, recent studies have shown that  $P_{50}$  and  $P_{88}$  values are not always correlated with  
102 mortality. It has been reported that conifers showing a percentage loss of conductance (PLC)  
103 of 80% (i.e. higher than 50%) and angiosperms with a PLC close to 100% (i.e. higher than 88%)  
104 are still able to recover from drought once irrigated [17–19]. These findings thus question the  
105 reliability of the  $P_{50}$  and  $P_{88}$  indicators for predicting tree death and, more importantly, it  
106 highlights the importance of determining the mechanistic link between xylem embolism and  
107 mortality in order to determine how hydraulic failure affects the capacity of trees to recover  
108 from drought. To explain the variation in PLC thresholds across individuals or species we argue  
109 that besides the level of embolism, the level of cell damage in the meristematic tissues should  
110 also be taken into account when evaluating a tree's capacity to recover from drought. Indeed,  
111 even though the cellular desiccation that results from hydraulic failure has been part of the

112 framework proposed by McDowell *et al.* [9,20], studies focused on demonstrating explicitly  
113 this link are virtually inexistent [21,22] and none have worked at the meristematic level. Thus,  
114 there are two fundamental aspects that have so far been considered independently but which  
115 must be brought together to properly identify, explain and understand the causes of drought  
116 dieback: (i) tree mortality is physiologically determined by meristematic cells vitality, and (ii)  
117 hydraulic failure is a strong determinant of drought-induced tree mortality. Only by considering  
118 both aspects together we will be able to elucidate the mechanistic links between hydraulic  
119 functioning, **cell death** and finally tree mortality. This opinion piece aims to shed the light on  
120 the mechanistic links between hydraulic failure and drought-induced tree mortality by  
121 providing a new approach on tree mortality that assumes that saving key meristematic cells  
122 from dehydration and allowing their rehydration are the two critical points for tree survival.  
123 This approach thus encourages new studies focused on cell water status, in particular at the  
124 meristems level to set new thresholds for predicting tree mortality with mechanistic models  
125 [23,24].

## 126 **Looking at cell death to predict tree death**

### 127 *Cell mortality under drought conditions: Membrane integrity matters*

128 One of the main causes of drought-induced tree mortality is the direct cellular  
129 consequences of dehydration ending in cell death [9,25]. Cell death in plants occurs by two  
130 different mechanisms: programmed cell death and necrotic cell death. **Necrosis** is the main  
131 process induced by a range of abiotic stresses (e.g. drought) [26]. While undergoing necrosis,  
132 cells usually present various anatomical features, such as swelling of the mitochondria [27],  
133 early rupture of the plasma membrane and/or shrinkage of the protoplast [26,28]. The loss of  
134 integrity of the membrane structure in necrotic cells and thus the early rupture of the plasma  
135 membrane have already been reported in plants exposed to frost due to dehydration preceding  
136 cell death [29]. Under fast dehydration, the cell's membrane changes from a liquid crystalline  
137 phase to a gel phase that can lead to a lateral phase separation of membrane constituents and  
138 cause membrane leakage [30–32]. Guadagno *et al.* [33] showed how the stability of the cellular  
139 membrane and thus its rupture was closely linked to a plant's water status. They proposed a  
140 theoretical mechanism of plant mortality based on membrane disruption as the most proximal  
141 cause of plant mortality under drought conditions. Recent observations of Mantova *et al.* have  
142 lent this theoretical mechanism some experimental support by showing how cell membrane  
143 integrity can work as an indicator for evaluating capacity to recover from drought in conifers  
144 [18]. To work towards elucidating the causes of drought-induced tree mortality and improve



145 our capacity to predict it, it is thus crucial to focus future research on the mechanisms and  
146 sequence of events associated with cell mortality and, especially with cell membrane failure.

147 *What fails? Focus on cell membrane failure*

148 Under drought conditions, membrane failure usually occurs by either structural changes  
149 related to the loss of solvation of the polar groupings of amphiphilic lipids [31] or to the  
150 biochemical modifications induced by the lipid peroxidation that results from the accumulation  
151 of **reactive oxygen species** (ROS) [34,35]. Abiotic stress such as drought increases the  
152 production and accumulation of ROS, leading to an oxidative stress [30, 31]. This ROS  
153 accumulation damages a broad variety of organic substances including the membrane  
154 components [34,35], in which it causes a modification of protein and lipid peroxidation leading  
155 to membrane leakage and consequently to cell lysis and cell death [33–35]. Looking at the  
156 survival strategy of plants able to support extreme dehydration levels, i.e. able to survive with  
157 low water content in their tissues (e.g. resurrection plants), ROS accumulation is counteracted  
158 by an increase in ROS-scavenging enzymes and antioxidant compounds [36,37], which prevent  
159 cell membrane disruption and maintain cell integrity [38]. ROS accumulation, and the altered  
160 cell redox status, is therefore an ubiquitous factor for cell mortality during water stress owing  
161 to its effects on cell membrane integrity, whence the importance of focusing future research on  
162 the relationship between drought-induced ROS accumulation, or more generally cell redox  
163 status, and cell membrane stability in perennial organs. Our hypothesis is therefore that cell  
164 ROS accumulation or the altered cell redox status are good candidates for identifying a  
165 threshold for cell mortality during drought, excluding the possible effects of pathogens and  
166 insects attacks, and to some extent for tissue vitality and more generally for organ and whole  
167 tree death (Figure 1, Key Figure), especially when evaluating meristematic tissues. There is  
168 thus an urgent need to consider both cell death and ROS accumulation or redox status of living  
169 cells crucial for recovery when seeking an indicator for tree mortality or lack of recovery  
170 capacity.

171 Despite the altered redox status of the cells has been demonstrated as one of the causes  
172 of membrane failure under drought conditions, not only biochemical processes but also  
173 different physical constraints could be also involved in the processes provoking cellular death.  
174 Indeed, **cellular cavitation** and **cell cytorrhysis** have been speculated as potential mechanisms  
175 driving cell mortality under water stress [39,40]. On the one hand, the cellular cavitation [41]  
176 could provoke lethal cellular injury by the rupture of the protoplasm when cell are exposed to  
177 important negatives pressures under frost conditions [39]. Similarly, when trees are exposed to

178 drought conditions and the water potential decreases progressively, cellular cavitation events  
179 could probably occur in different cell types including the meristematic ones. On the other hand,  
180 the cytorrhysis and collapse of the cell [37,42] has also been describe in previous studies in  
181 cells undergoing a water-stress [39,43] and could represent a major form of cell injury and,  
182 consequently, meristems damage in trees under severe drought conditions. However, whether  
183 cellular cavitation and cytorrhysis provoke cellular death by membrane disruption is, to date,  
184 unknown and would require experimental studies including microscopy techniques, such as  
185 Cryo-SEM, to visualize both the cell shape and plasma membrane integrity. Whether those  
186 physical constraints occur at the meristems level and could work as an indicator for tree  
187 mortality or capacity of recovery is still unresolved and would require further studies targeting  
188 especially those key tissues for tree survival (Figure 1). Therefore, whether these physical  
189 processes are mutually exclusive of the oxidative stress is still unknown.

#### 190 **Tree water relations and meristematic tissues mortality**

191 In the meristematic approach proposed for assessing drought-induced dieback,  
192 meristematic elements (e.g. primary and secondary meristems) are the key elements for  
193 evaluating a tree's capacity to survive intense drought episodes [44–46]. During drought, it has  
194 been shown how roots undergo progressive contraction and rupture of cambial cells leading to  
195 their disintegration in severely water-stressed and non-recovering seedlings [46] highlighting  
196 the relevance of these meristematic cells [47,48] when evaluating tree senescence and death.  
197 Besides the meristematic tissues, it is also important to consider the relevance of other cell types  
198 such as cortical parenchyma, that may dedifferentiate to produce 'adventitious' meristems once  
199 the meristematic cells have lost their capacity to differentiate and develop into other tree tissues  
200 and organs [49,50]. However, how the capacity of the meristematic cells to differentiate and to  
201 the other cell type (e.g. parenchyma cells) to dedifferentiate is affected by the progressive  
202 reduction in plant hydraulic functioning and the dehydration of the tissues, is largely unknown  
203 and deserves further attention from both physiological and ecological points of view.

204 In plants undergoing a drought resulting in rapid extreme dehydration, cells also  
205 undergo water stress by reduced **relative water content** (RWC), which can eventually induce  
206 cell death [51]. Meristematic cells will thus also start to dehydrate and enter the cell death phase  
207 [36], displaying evidence of necrosis as they approach their critical water status. It is therefore  
208 to be expected that there will be a threshold in RWC below which these cells will show evidence  
209 of dehydration-induced necrosis. However, previous studies on the critical water status  
210 allowing cell survival under water stress conditions have highlighted that the critical water

211 status for survival varies among cell types, organs and species. This is the case for some seed  
212 types able to keep their capacity to germinate up to RWC values of ca. 22% [52], whereas the  
213 critical RWC for leaf survival ranges between 7.0% and 58.5% depending on the species [22].  
214 Like leaves, most mesophytic plants cannot withstand an RWC below 50% [53]. Reaching  
215 critical RWC in meristematic tissues will therefore affect the capacity of a whole tree to recover  
216 from drought. This RWC threshold, however, may vary across species according to their  
217 resistance to drought and to their mechanisms to protect cells from dehydration (e.g. production  
218 of late embryogenesis abundant proteins in seeds [37]). Using RWC, that has been recently  
219 presented as a more mechanistically relevant metric of plant lethal water stress [54,55], as a  
220 threshold for meristematic cells mortality and thus tree recovery capacity would greatly  
221 improve mechanistic models aimed to predict and anticipate the resilience of trees after drought  
222 events, a crucial step in forecasting catastrophic forest dieback [23,24] (Figure 1). As membrane  
223 integrity seems a good indicator of cell vitality and is related to cell water content [29,33,56,57],  
224 a focus on the sequence of events taking meristematic cells into necrosis with regard to RWC  
225 could set a physiological threshold for meristematic cells death and thus tree survival (Figure 1).  
226 Also, as RWC, oxidative stress and physical constraints are inter-related, ascertaining which  
227 threshold is prominent would help precisely determine the physiological threshold for  
228 meristematic cells beyond which they lose their ability to differentiate. The time at which  
229 different species reach these threshold levels will therefore depend on both their dehydration  
230 rates during drought [58] and on their resistance to drought according to their mechanisms to  
231 avoid oxidative stress [37]. Describing the main processes taking meristematic cells to critical  
232 dehydration levels during drought, how it varies across organs (bole, branch tips, roots) and  
233 species and how they all interact with each other – including hydraulic functioning, residual  
234 conductance, and capacitance of the different tissues and organs – is a promising future research  
235 direction towards identifying the mechanistic processes underlying drought-induced tree  
236 mortality.

### 237 *Hydraulic functioning and dysfunctioning*

238         The water content of any organ or tissue of a tree is determined by the balance between  
239 water loss and absorption. Water losses are mainly due to stomatal or residual transpiration  
240 [59,60] and are therefore imposed by microclimatic conditions. Water absorption depends  
241 mainly on the ability of trees to extract water from the soil and transport it upwards to the  
242 meristematic cells (Figure 2). Thus under drought conditions, the increasing environmental  
243 evaporative demand causes an increasing tension in the xylem, inducing cavitation events and

244 finally the hydraulic failure of the water transport system when it undergoes a runaway effect  
245 leading to complete loss of its conductance [61]. This dysfunction of the water transport system  
246 causes a significant reduction in the amount of water supplied to the tree's living tissues, which  
247 will accordingly decrease their RWC, increase the percentage of water-stressed cells and finally  
248 trigger cell necrosis processes (Figure 2) [22]. While McDowell *et al.* (2008) has already  
249 mentioned that hydraulic failure leads to cellular death, little is known on the desiccation of the  
250 actual meristems, probably because of the difficulty to access those elements. However, the  
251 desiccation of the meristems could provide us with the mechanical link between hydraulic  
252 failure and tree mortality [17,18]. Thus, as significant correlations between high levels of PLC  
253 and tree mortality have been reported for several species [6,7,10], the central hypothesis we  
254 advance here is that the hydraulic failure of the xylem is the triggering factor in the irreversible  
255 dehydration of meristematic cells, severely impairing their water absorption capacity and thus  
256 their water content, so leading to their death. A reduced RWC resulting from hydraulic failure  
257 should therefore be ubiquitous to all tree tissues independently of their function [22,54,55,62]  
258 and would certainly be found at meristematic tissues level causing cells death and ultimately  
259 the inability of the tree to recover from drought [9,25] (Figures 2, 3). However, accessing the  
260 meristematic tissues might be challenging in practice. Therefore, as a first step, the sampling of  
261 buds would allow to efficiently link the hydraulic failure of the xylem with meristems mortality.  
262 Then, once this link is evaluated, accessing cambial cells using e.g. transmission electron  
263 microscopy would allow to make a significant step forward in the understanding of tree death  
264 from drought [46]. As RWC thresholds have the potential to be use for remote sensing tree  
265 mortality [63,64] another step forward would be to observe how meristems RWC might relate  
266 to canopy moisture content [65,66] to be able to predict more accurately the consequences of  
267 drought on trees' survival at the population and the landscape level.

268         Recent studies have reported tree mortality events when the losses in stem xylem  
269 conductance were higher than the proposed PLC thresholds of 50% and 88% for conifers and  
270 angiosperms, respectively and that the PLC value provoking tree death likely varies across  
271 individuals and species [17,18,67]. This has thus highlighted the importance of (i) revisiting the  
272  $P_{88}$  and  $P_{50}$  thresholds and (ii) the need to determine more accurately the PLC leading to the  
273 runaway dehydration of meristematic cells. Under hydraulic failure conditions, the water lost  
274 through transpiration will not be compensated by the water entries, and meristematic tissues  
275 will ultimately dehydrate, desiccate and die (Figure 3). As suggested by Hammond *et al.* [17],  
276 there should thus be a sequence of events occurring on reaching significant losses in hydraulic

277 functioning, and leading to meristems and tree death (Figures 1, 3). Along this sequence, we  
278 hypothesize that the cell metabolism would depend first on the tree's water reserves at the  
279 **apoplastic** level, and once this water is depleted, on their own water content (**symplastic**). As  
280 this is in turn exhausted, meristematic cells will undergo dehydration and death by necrosis [9]  
281 (Figure 1). Thus to validate our hypothesis, evidence of meristematic cell necrosis should  
282 appear after high losses in plant hydraulic functioning that would alter the water status of the  
283 meristems (Figure 3). As meristem water content depends to a large extent on tree water supply  
284 related to tree hydraulic functioning, a better understanding is needed of the minimal tree  
285 hydraulic conductance required to keep the meristematic tissues hydrated and so allow the tree's  
286 recovery from drought. What matters is (i) whether meristematic cell dehydration can be  
287 delayed through the radial relocation of water within the stem during drought [68–70] and  
288 (ii) under what conditions the cell can rehydrate once re-supplied with water.

#### 289 *Avoiding meristematic cells mortality*

290 As already described, xylem hydraulic failure disrupts the water supply to a tree's living  
291 tissues [71] and forces downstream organs to rely on their own water reserves (symplastic) [72].  
292 Although plant water storage has been widely studied in terms of localisation [68,73], the link  
293 between water release and hydraulic failure, mobilisation to maintain the metabolism of  
294 meristematic cells, and possible refilling after a drought event, have been poorly evaluated [69].  
295 In general, there are two main types of water storage reservoirs in plants: symplastic reservoirs  
296 (i.e. inside the living cells) and apoplastic reservoirs (Figure 1) [68,73]. The water stored in  
297 these two reservoirs can be mobilised during drought as tree's water potential decreases [73,74].  
298 In general, there is a bidirectional transport of water between inner bark and xylem in  
299 angiosperms through the symplasmic space of ray parenchyma cells. Thus, water moves from  
300 phloem to the mature xylem via the parenchyma cells and the cambial zone [70]. As the  
301 formation of emboli in the xylem is initiated, the apoplastic water pools seems to be mobilised  
302 to preserve living tissue (i.e. symplastic compartments) hydration state rather than to buffer  
303 xylem cavitation [73,75]. Indeed, as demonstrated with X-ray micro-CT, the water contained  
304 in the xylem matrix embedding xylem vessels (i.e. mainly fibres) is released to the adjacent  
305 tissues concomitantly to stem shrinkage and the formation of embolism, and not before the  
306 onset of cavitation [73,75]. As the xylem tension increases, more cavitation events are observed  
307 in those xylem vessels located close to the vascular cambium, along with more air-filled cells  
308 within the xylem matrix [75]. This suggests that the mobilisation of these water reserves could  
309 partially buffer the water depletion in the symplastic reservoirs that could include key living

310 tissues for tree survival (Figure 1) [69,75]. By bringing together all these recent results, it can  
311 be hypothesized that water released from the xylem matrix (apoplastic compartment) is moved  
312 to the adjacent tissues and consequently to meristematic tissues (symplastic compartment) to  
313 keep their cells metabolically active. Once all the water stored in both the apoplastic and the  
314 symplastic compartments has been released, tree living tissues and cells will ultimately dry out,  
315 and meristematic cells will suffer from mainly oxidative stress, cavitation and cytorrhysis and  
316 finally die (Figure 1). However, there are still significant open questions about the possible role  
317 of the water supplied from the bark in protecting the cambial cells from dehydration. Testing  
318 this hypothesis will require new experimental studies focused specifically on describing the  
319 processes of water release and water relocation, their mechanistic link with hydraulic  
320 functioning, and the sequence and timing of the events and processes occurring in the apoplastic  
321 and symplastic compartments to protect the meristematic cells during drought [68,76].

## 322 **Concluding remarks and future perspectives**

323 Tree mortality is physiologically determined by meristematic cells vitality. However,  
324 the consequences of drought on these cells and the subsequent recovery of the trees are still  
325 under-researched. As different recent studies have identified a significant correlation between  
326 losses in hydraulic functioning and drought-induced tree mortality, it is now crucial to  
327 determine the mechanistic link between these two processes by evaluating the effect of  
328 hydraulic failure on the water content of the different tree organs and tissues, and especially on  
329 the meristematic cells. In addition, evaluating meristematic cell integrity with regard to RWC,  
330 redox status and physical constraints would provide novel and valuable information about the  
331 physiological thresholds determining cell mortality, which would be most useful when  
332 implementing mechanistic models aimed at predicting tree mortality and so forest dieback  
333 under drought conditions (Figure 1). Following the dynamic of dehydration of the meristematic  
334 cells from the beginning of tree dehydration and especially once the tree has reached xylem  
335 hydraulic failure at stem level is central to addressing key physiological questions related to  
336 drought-induced mortality, such as:

- 337 (i) Whether there is a mechanistic link between xylem cavitation, meristems water  
338 content, ROS accumulation and membrane stability.
- 339 (ii) Whether there is any critical threshold in RWC and/or oxidative stress and/or  
340 physical constraints from which the meristematic cells begin to show significant  
341 damage, and whether they are influenced by the duration and intensity of the  
342 drought.

- 343 (iii) Whether trees have evolved a water relocation strategy to protect key plant tissues  
344 from dehydration to maintain their capacity to recover from drought.

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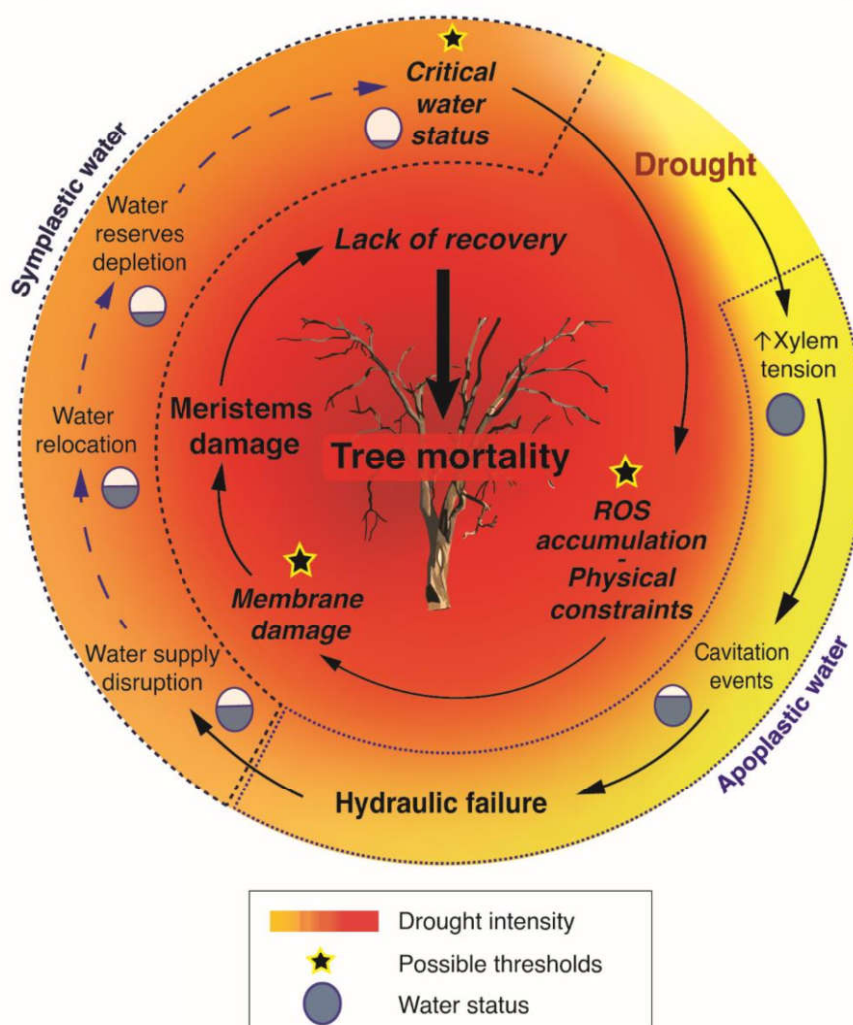
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**Integrative framework representing the main processes explaining the correlation between hydraulic failure and tree mortality**



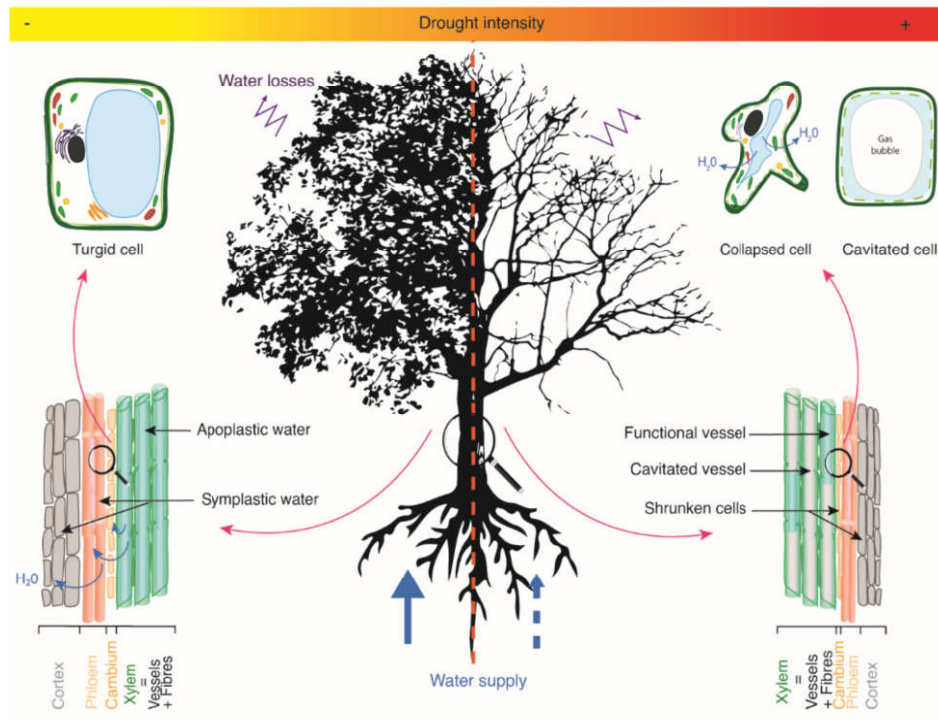
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**Figure 1, Key Figure. Integrative framework representing the main processes explaining the correlation between hydraulic failure and tree mortality**

Drought-induced tree mortality is currently predicted with mechanistic models using thresholds that mark a point of no return from drought. Although there is a strong correlation between a high loss of conductance in trees ( $P_{50}$  for conifers and  $P_{88}$  for angiosperms) and their mortality, some trees can still survive drought after high losses in their hydraulic capacity. This new approach emphasises the importance of studying the link between the disruption of cell water supply due to xylem hydraulic failure and a change of water status in the meristematic cells. It also shows the consequences of a critical water status on the production of reactive oxygen species (ROS) as well as changes in the physical constraints on the cells, which can cause severe

533 membrane damage in the meristematic cells and eventually induce their death. This framework  
534 thus presents the new possible thresholds that, according to the different hypotheses presented  
535 in the text, could be used to implement mechanistic models for predicting tree mortality in the  
536 context of climate change. In this figure, the arrows represent the sequence of event leading to  
537 tree mortality. The dashed blue arrow represent the hypothetical sequence of event requiring  
538 further studies.

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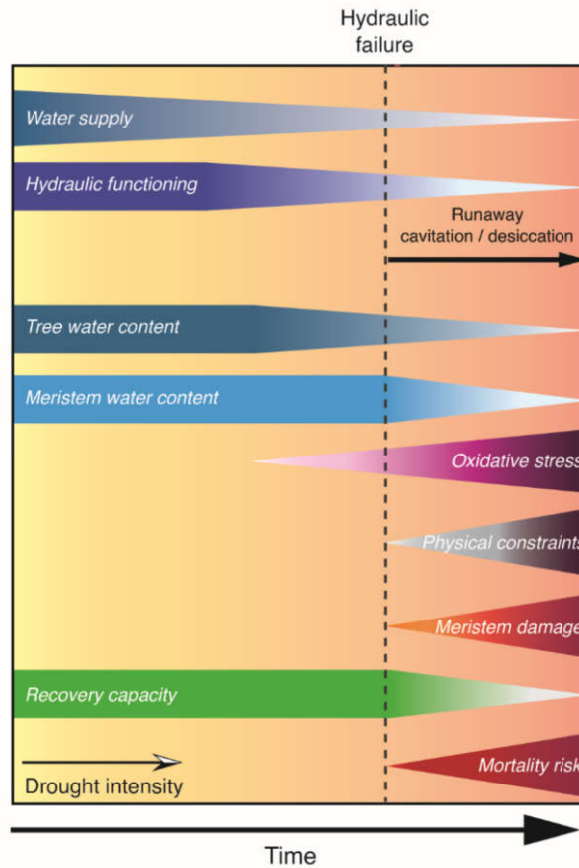
542 **Figure 2. Consequences of water stress on tree water transport and tree cells**

543 Under mild water stress conditions, trees are able to keep their hydraulic system almost fully  
 544 functional, and water is continuously transferred from the xylem (apoplastic water) to the cells  
 545 in the living reservoir (symplastic water) maintaining the turgidity and integrity of the cells.

546 As water stress is exacerbated, the loss of hydraulic conductance in the xylem pathway (PLC)  
 547 increases until it causes xylem hydraulic failure, when the water supply cannot meet the tree's  
 548 water requirements. Concomitantly to the loss of xylem conductance, water transport from the  
 549 xylem to the living tissues (e.g. cortex and cambium) is disrupted and the metabolism of cells  
 550 relies first on water supply and finally on their own water reserves. Consequently, cells start to  
 551 dehydrate, shrink, collapse or cavitate and finally die from necrosis when they lose their  
 552 membrane integrity.

553





554

555 **Figure 3. Drought consequences on tree water transport and content**

556 As drought intensity is exacerbated (from yellow to orange) and water supply decreases, xylem  
 557 hydraulic functioning decreases until it reaches hydraulic failure, when water supply no longer  
 558 meets water demand. Concomitantly to the loss of hydraulic functioning, tree water content  
 559 diminishes and causes the dehydration of the meristems and an increase in oxidative stress.  
 560 After reaching xylem hydraulic failure, meristematic cells enter the phase of irreversible  
 561 dehydration and finally incur damage, particularly at membrane level. Consequently, the  
 562 recovery capacity of the tree diminishes, and the mortality risk increases. Quantifying  
 563 meristematic cell water content at the time of increasing cell damage (i.e. evidence of meristems  
 564 damage) (black dashed line) could identify thresholds below which the tree will be unable to  
 565 recover from drought and will ultimately die.

566 **Box 1. Drought-induced mortality: Dying of thirst**

567 Drought-induced tree mortality is related to drought intensity, duration and frequency [9].  
568 Under extreme drought conditions (i.e. long intense droughts), tree mortality mostly arises from  
569 xylem hydraulic failure [10,14].

570 During a severe drought event, soil water availability decreases while evaporative demand and  
571 cuticle conductance increase, resulting in an increment of the xylem tension that induces the  
572 occurrence of cavitation events in the xylem. Cavitation is the change from liquid water to water  
573 vapour under increased tension. This change in water phase results in the formation of gas  
574 bubbles termed ‘emboli’ in xylem conduits [77,78]. The cavitation of one xylem element can  
575 spread throughout the xylem vessel [71] when xylem tension increases owing to an increased  
576 vapour pressure deficit. As the percentage of cavitared vessels increases, the hydraulic  
577 conductance of the xylem decreases until the flow of water stops and causes the dehydration of  
578 the tree tissues, cell death, and the death of the tree.

579 Vulnerability to cavitation is extremely variable across species and biomes [13,14] and is  
580 usually evaluated by constructing vulnerability curves representing the percentage loss of  
581 hydraulic conductance (PLC) induced by cavitation with regard to xylem tension (i.e. xylem  
582 water potential). From these curves are extracted the xylem tension inducing 50% loss of  
583 hydraulic conductance ( $P_{50}$ ) and that inducing 88% loss of hydraulic conductance ( $P_{88}$ ).  $P_{50}$  is  
584 generally used as an indicator of tree resistance to cavitation. When modelling tree survival  
585 from drought,  $P_{50}$  and  $P_{88}$  have been used, so far, as lethal thresholds for conifers and  
586 angiosperms respectively [15,16]. However, recent experimental results do not always support  
587 both of these numbers and particularly  $P_{50}$  [17,18] which emphasize the need to consider other  
588 physiological traits when trying to define a mortality threshold.

589

590