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Marylou Mantova, Stéphane Herbette, Hervé Cochard, Jose Manuel Torres Ruiz

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TITLE

Hydraulic failure and tree mortality: From correlation to causation

AUTHOR NAMES

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

HIGHLIGHTS

- 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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OUTSTANDING QUESTIONS

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

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Opinion article

TITLE

Hydraulic failure and tree mortality: From correlation to causation

AUTHOR NAMES

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

Université Clermont Auvergne, INRAE, PIAF, 63000 Clermont-Ferrand, France

*Correspondence: torresruizjm@gmail.com (José M. Torres-Ruiz)

ORCID

Marylou Mantova: https://orcid.org/0000-0003-4445-3100

Stéphane Herbette: https://orcid.org/0000-0002-1226-6071

Hervé Cochard: https://orcid.org/0000-0002-2727-7072

José M. Torres-Ruiz: https://orcid.org/0000-0003-1367-7056

TWITTER

https://twitter.com/MarylouMantova

https://twitter.com/StephaneHerbet1

https://twitter.com/HCochard

https://twitter.com/torresruizjm

KEYWORDS

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

GLOSSARY

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

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

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

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

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

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

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

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

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

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

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

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

ABSTRACT

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

MAIN TEXT

Dying of thirst

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

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

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

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

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

What fails? Focus on cell membrane failure

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

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

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

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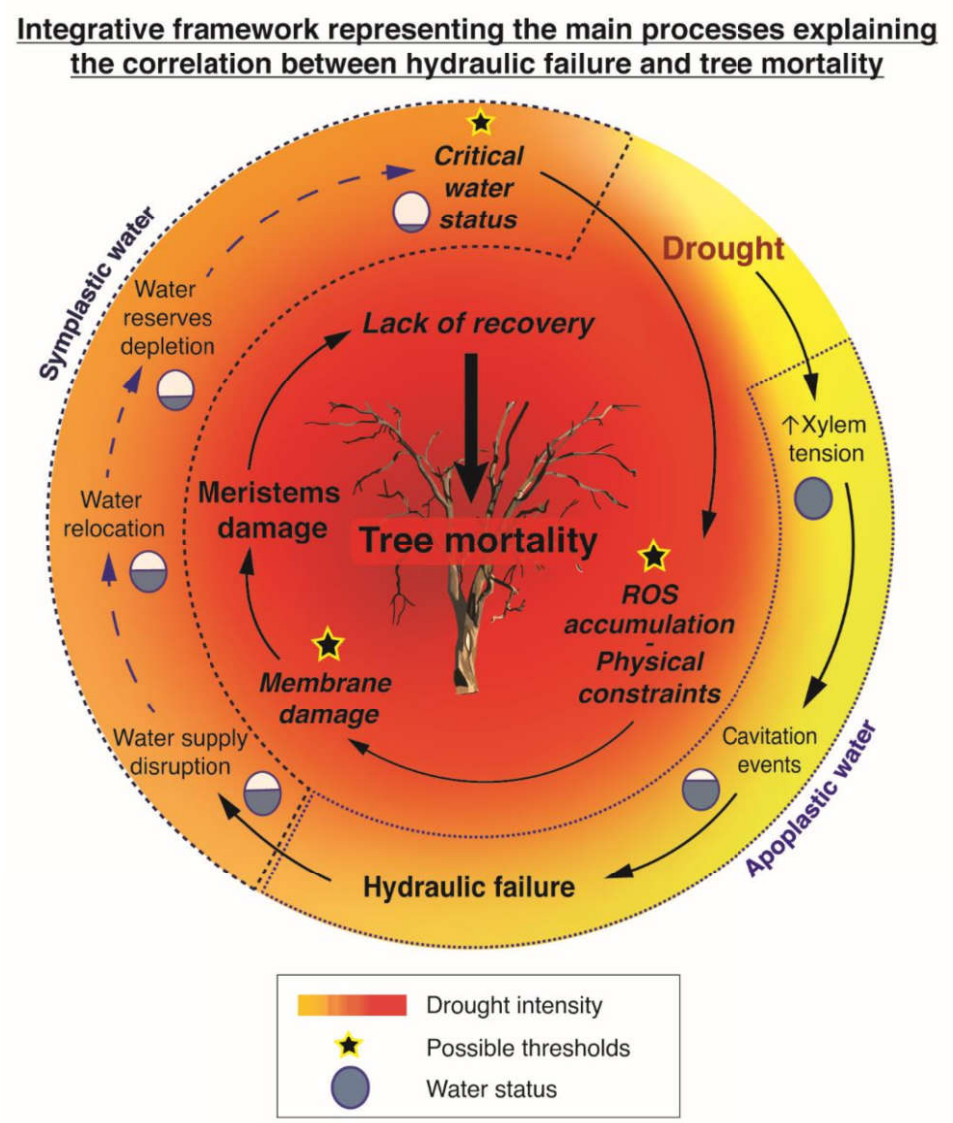

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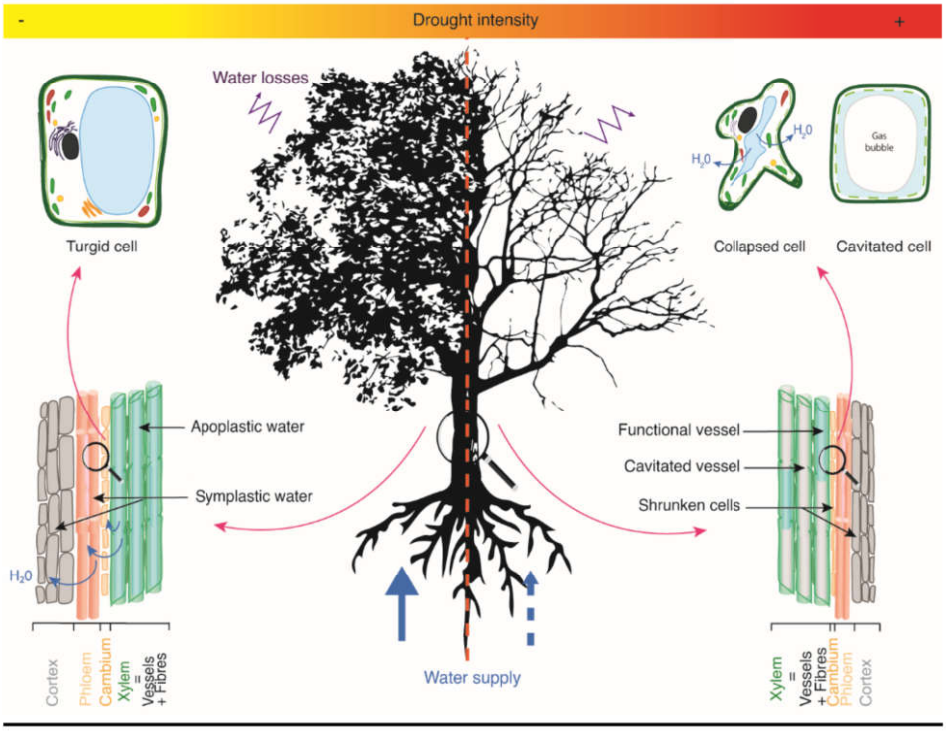
523 **<u>Figure 1, Key Figure</u>. Integrative framework representing the main processes explaining**
524 **the correlation between hydraulic failure and tree mortality**

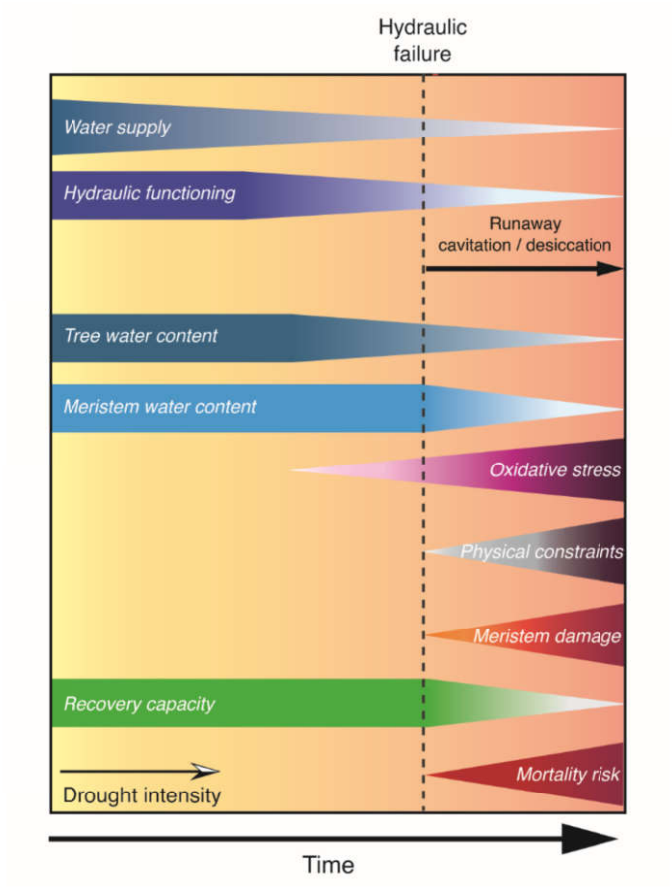
525 Drought-induced tree mortality is currently predicted with mechanistic models using thresholds
526 that mark a point of no return from drought. Although there is a strong correlation between a
527 high loss of conductance in trees (P_{50} for conifers and P_{88} for angiosperms) and their mortality,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

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



<u>Figure 2.</u> Consequences of water stress on tree water transport and tree cells

Under mild water stress conditions, trees are able to keep their hydraulic system almost fully functional, and water is continuously transferred from the xylem (apoplastic water) to the cells in the living reservoir (symplastic water) maintaining the turgidity and integrity of the cells. As water stress is exacerbated, the loss of hydraulic conductance in the xylem pathway (PLC) increases until it causes xylem hydraulic failure, when the water supply cannot meet the tree's water requirements. Concomitantly to the loss of xylem conductance, water transport from the xylem to the living tissues (e.g. cortex and cambium) is disrupted and the metabolism of cells relies first on water supply and finally on their own water reserves. Consequently, cells start to dehydrate, shrink, collapse or cavitate and finally die from necrosis when they lose their membrane integrity.



<u>Figure 3.</u> Drought consequences on tree water transport and content

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

566 **<u>Box 1.</u> 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 cavitated 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

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