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1 **Threats to xylem hydraulic function of trees under 'new climate normal' conditions**

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3 Maciej A. Zwieniecki and Francesca Secchi

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5 Department of Plant Sciences, UC Davis, Davis CA 95616

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7 Corresponding author: Maciej A. Zwieniecki,

8 University of California – Davis, Department of Plant Sciences, One Shield Avenue, Davis, CA,

9 95616

10 Email: mzwienie@ucdavis.edu

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12   **Abstract**

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14   Climate models predict increases in frequency and intensity of extreme environmental

15   conditions, such as changes to minimum and maximum temperatures, duration of drought

16   periods, intensity of rainfall/snowfall events, and wind strength. These local extremes, rather

17   than average climatic conditions, are closely linked to woody plant survival, as trees cope with

18   such events over long lifespans. While xylem provides trees with structural strength and is

19   considered the most robust part of a tree's structure, it is also the most physiologically vulnerable

20   since tree survival depends on its ability to sustain water supply to the tree crown under variable

21   environmental conditions. Many structural, functional, and biological tree properties evolved to

22   protect xylem from loss of transport function due to embolism or to restore xylem transport

23   capacity following embolism formation. How 'the new climate normal' conditions will affect

24   these evolved strategies is yet to be seen. Our understanding of xylem physiology and current

25   conceptual models describing embolism formation and plant recovery from water stress,

26   however, can provide insight into near future challenges that woody plants will face. In addition,

27   knowledge of species-specific properties of xylem function may help guide mitigation of climate

28   change impacts on woody plants in natural and agricultural tree communities.

29

Introduction

Trees are long-lived organisms, anchored in one place for tens, hundreds, or thousands of years. They rely on a vascular transport system that, although continuously renewed via annual xylem and phloem growth, must endure local climatic extremes including events of variable frequency, intensity, and duration. Global climate models predict that the small changes in recent climate observed across the planet are only a prelude to more dramatic changes in the near future, such as prolonged drought periods and increased frequency of frost events (IPCC, 2013, (Bloom, 2010, Hayhoe *et al.*, 2004). Changing frequency or intensity of extreme events might overwhelm the adaptive limits of vascular transport, resulting in continued periods of vascular function loss and increased incidence of tree mortality. In fact, we already observe increased mortality of trees in natural ecosystems stretching across multiple regions around the world that are being linked to drought and carbohydrate starvation (Hartmann *et al.*, 2013, O'Grady *et al.*, 2013, Sevanto *et al.*, 2014). Not only are natural ecosystems in danger, the same fate might be expected for the perennial woody crop plants in orchards. Despite increased agro-engineering efforts, we are limited in our ability to protect orchard trees from global scale consequences of climate change, including loss of access to fresh water, increased salinization of agricultural soils, and rising atmospheric temperature. In addition, orchards are planted today with the expectation of continued productivity for the next 20-40 years, an expectation that might be increasingly difficult to achieve under predicted climate change scenarios (Lobell *et al.*, 2006). To mediate potential damage to agricultural investments and protect natural forest ecosystems, we should better understand how "new climate normal" conditions will influence critical aspects of vascular physiology, namely vascular embolism formation and recovery.

Embolism

Long-distance water transport in vascular plants occurs in the xylem, a network of nonliving cells connecting roots to leaves (Sperry *et al.*, 2003). Typically, the apoplastic water column in functional xylem is under tension and considered to be in metastable state (Stroock *et al.*, 2014). When internal tension reaches a critical threshold at which the conduit radius is larger than the critical radius of the vacuum/water interface (critical radius $r_{critical}$ being defined as equality between meniscus force defined as water surface tension (γ) divided by air bubble radius) that could oppose expansion due to water surface tension, the conduit water column can break forming an embolus, a phenomenon referred by many plant biologists as 'cavitation' (Tyree & Zimmermann, 2002). We note that cavitation (considered as spontaneous initiation of vapor bubbles in pure metastable water) is a highly improbable event in xylem, as it occurs spontaneously at pressures far more negative [i.e. below -140 MPa (Brenner, 1995)] than are physiologically relevant (i.e. above -20 MPa). Thus, we will abstain from using word 'cavitation' and only refer to the process as 'embolization' or 'formation of embolism'. Despite the very low probability of true spontaneous cavitation in stems of trees, it was shown using multiple indirect and direct methods that xylem conduits (vessels and tracheids) can switch from a water filled to an embolized state even under tension as low as 1 MPa (Brodersen *et al.*, 2010, Holbrook *et al.*, 2001, Zwieniecki *et al.*, 2013). To reconcile the low probability of spontaneous cavitation with observations of naturally occurring embolism levels, an "air-seeding" hypothesis was proposed as a possible mechanism to explain formation of embolism in xylem under relatively low tensions (Zimmermann, 1983).

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"Air-seeding" is assumed to have several potential origins that are not mutually exclusive, all of which can influence the level and threshold for xylem embolism formation (Capron *et al.*, 2014, Zimmermann, 1983). All proposed explanations assume that somewhere along the conduit an air-water interface exists with a radius that is smaller than the expansion threshold (Figure 1). This interface can exist across the inter-vessel connection (bordered pit field) (Lens *et al.*, 2013), in the crevices of the vessels or tracheid (Pickard, 1981, Zimmermann, 1983), or simply on the wall surface where stable bubbles persist for a prolonged time (Weijs & Lohse, 2013). Increasing tension would result in the change of the critical radius required to overcome water surface tension such that the bubble slowly changes shape and expands into the conduit eventually reaching a critical radius and 'explodes' into the conduit causing an embolism that effectively ceases water transport as tension cannot be transmitted via gas. This type of embolism formation might be considered a spatially and temporally unpredictable phenomenon or it might be related to the degree of tension, thermal environment, xylem physical properties, water chemical properties, and history of previous embolism activity (Hacke *et al.*, 2001, Holbrook & Zwieniecki, 1999, Stiller & Sperry, 2002, Tyree & Zimmermann, 2002). In addition to purely tension driven embolism, another origin of the embolism results from freeze-thaw events, where ice formation may lead to release of air from water which form small gas bubbles in the frozen liquid (Mayr *et al.*, 2007, Sperry & Sullivan, 1992). The fate of air bubbles during thawing depends on their size and the amount of tension in the xylem during thawing. If the air bubble radius is smaller than the critical radius for expansion and pressure differential between air in the bubble and water ($2\gamma/r_{critical}=P_{air}-P_{water}$), the air will dissolve in water and the bubble will either collapse or attach to the conduit wall forming a future "air-seeding" site. Alternatively, if

99   the air bubble radius is larger than the critical radius, the bubble will expand and an embolus will

100   form.

101   Regardless of embolism origin, the result is a reduction in the plant's capacity to transport water

102   and a loss of plant productivity or even plant death (Domec *et al.*, 2006b, Holtta *et al.*, 2009,

103   Tyree & Sperry, 1989, Zwieniecki & Holbrook, 2009). Therefore, a plant's capacity to reduce

104   the detrimental effects of embolism is an important adaptive trait for growth and survival

105   (Barigah *et al.*, 2013, Choat *et al.*, 2012, Jacobsen *et al.*, 2007, Pockman *et al.*, 1995, Tyree &

106   Ewers, 1991) and can be affected by climatic properties, especially temperature and moisture.

107   Resistance to drought and frost-induced embolism are important adaptive traits for defining the

108   limits of embolism tolerance across woody species and for predicting drought-induced forest

109   decline at regional and global scales (Choat *et al.*, 2012, Lens *et al.*, 2013). To better understand

110   the role of climate in this system we need to look at the anatomy and associated physicochemical

111   properties of xylem including (i) the structure of the pit membranes, (ii) diameter and length of

112   conduits and (iii) wood density within the context of major climate variables.

113

114   *Pit membranes.*

115

116   Bordered pits are cavities in the lignified cell walls of adjacent vessel or tracheid conduits. They

117   are a pivotal component in the water-transport system of seed plants. Bordered pits are not

118   simple holes that allow water passage between cells. They are highly engineered structures that

119   are strongly conserved among species and that are responsible for more than 50% of total xylem

120   hydraulic resistance (Choat *et al.*, 2008, Hacke *et al.*, 2006, Wheeler *et al.*, 2005) indicating that

121   they are an important factor in the overall hydraulic efficiency of plants and may be fundamental

122   to species survival. In general, bordered pits are formed as circular openings with species-

123   specific diameters ranging from 1 to 20 µm, protected on both ends by a bowl shaped wall,

124   typically with a smaller opening leading to the pit. The pit membrane is located at the center

125   where it separates adjacent conduits and mediates two seemingly contradictory functions: (1)

126   allowing water to flow between adjacent conduits and (2) protecting conduits against the spread

127   of embolism (Choat *et al.*, 2008, Tyree & Sperry, 1989).

128   The structure of pits and pit membranes varies among species, with differences in pit diameter,

129   pit depth, wall smoothness, membrane porosity, membrane thickness, and membrane total

130   surface area. For example, among 26 angiosperm species collected from a range of provenances

131   including riparian, temperate, and Mediterranean environments, the pit membrane thickness

132   range from 70 to 1900 nm and pore sizes within the membrane from 10 to 225 nm (Jansen *et al.*,

133   2008). Species characterized by thicker pit membranes usually also have smaller pores and thus

134   are better adapted to protection from embolism spread as radius of the pores is inversely related

135   to critical tension sustained at the air/water interface. Jansen et al. (2008) also found a negative

136   relationship between pit membrane thickness and maximum pore diameter. In addition, pore

137   size in a given species is variable with species specific probability of large pores. As larger pores

138   allow embolism to spread more easily from conduit to conduit, the species with bigger pores or

139   with higher probability of their presence due to a larger membrane area between conduits will be

140   more vulnerable to vascular dysfunction (Choat *et al.*, 2008). Interestingly, there was no

141   correlation between hydraulic resistance of the pit membrane and vulnerability to embolism

142   across a wide range of species. Species with lower hydraulic resistance and higher average

143   porosity were not necessarily more vulnerable to embolism (Hacke *et al.*, 2006). This

144   discrepancy could be explained by the fact that it takes only one large pore in one membrane to

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145 spread embolism from conduit to conduit. As such big pores are relatively rare and might be

146 considered a membrane developmental error or could be a result of damage (Choat, 2013).

147 Increased cavitation risk may therefore be linked to total pitted area of xylem conduits, making

148 vessels with greater total area of pits more vulnerable to embolism (Jarbeau *et al.*, 1995). In

149 most gymnosperms, the bordered pit membrane is characterized by a porous margo and central

150 thickening (torus), which is considered to function as an impermeable safety valve against air-

151 seeding. Embolism resistance of conifer pits is correlated to the ratio of torus size versus pit

152 aperture diameter, suggesting a dependency on the torus' ability to seal the pit aperture (Domec

153 *et al.*, 2006a). Embolism resistant species showed higher margo flexibility and larger overlap

154 between the torus and pit aperture, suggesting that torus adhesion to the pit border may be a

155 determinant of cavitation resistance (Delzon *et al.*, 2010). In some conifer species the presence

156 of plasmodesmatal pores fields in the tori could contribute to air-seeding through them (Jansen *et*

157 *al.*, 2012) reducing effectiveness of the pore seal.

158

159 Overall, bordered pit membranes whether in gymnosperms or angiosperm trees seem to be the

160 first line of defense against conduit-to-conduit spread of embolism. A plasticity of bordered pit

161 morphology related to embolism formation was reported for Douglas-fir trees. It was shown that

162 in the branches and trunks the pit aperture diameter of tracheids decreases significantly with

163 increasing height, whereas torus diameter remains unchanged. The resulting increase in the ratio

164 of torus to pit aperture diameter provides better overlap of torus with pit aperture, resulting in the

165 capacity to sustain higher tensions before air-seeding (Domec *et al.*, 2008). There is also a

166 general pattern of smaller pits with thicker, less porous pit membranes in trees growing in drier

167 climates. However, almost nothing is known regarding the plasticity of bordered pit

168   morphological properties over the lifespan of a tree and whether the plant can respond to climatic

169   change. Specifically, it is not known if drier years trigger development of thicker pit membranes

170   and/or smaller pit areas within the same tree across multiple rings, ultimately leading to reduced

171   membrane porosity. This lack of knowledge leaves us unable to predict if trees growing today

172   can acclimate to changes in climate via developmental adjustment of bordered pit structure.

173   However, assuming that bordered pits evolved to protect xylem from embolism spread during

174   extreme events in the current climate, it is conceivable that increased frequency of extreme

175   weather events with more adverse conditions might lead to higher frequency of bordered pit

176   failures and greater incidence of embolism formation.

177

178   *Diameter and length of conduits*

179

180   Angiosperm and gymnosperm conducting elements differ in both diameter and length. Vessels

181   are bigger (length: 1-1000+ cm, diameter: 15-500 µm) than tracheids (length: 0.1-1.0 cm,

182   diameter: 5-80 µm) although the specific distribution of vessel lengths is not known (Choat *et*

183   *al.*, 2008, Zimmermann, 1983). Multiple lines of evidence suggest that wide and long conduits

184   tend to embolize before narrow conduits (Lo Gullo & Salleo, 1991, Lo Gullo *et al.*, 1995, Sperry

185   & Tyree, 1990). This may simply be related to the fact that conduits may contain more bordered

186   pit pores with larger total pit membrane area and thus higher probability of embolism spread due

187   to damage, malfunction, or developmental error (pit area hypothesis) (Sperry *et al.*, 2005, Tyree

188   *et al.*, 1994, Wheeler *et al.*, 2005). Indeed a strong correlation was found between conduit

189   diameter and embolism caused by freeze-thaw cycles (Davis *et al.*, 1999). However, analysis of

190   anatomical traits of xylem plasticity to water stress is more complex (Tyree *et al.*, 1994) as trees

191 were shown to plastically adjust their xylem anatomical traits (diameter and length of the

192 conduits) to drought. These adjustments are not straightforward as plants have to accommodate

193 contradicting demands, namely transport capacity and resistance to embolism formation. For

194 example, *Pinus sylvestris* trees grown in dry regions were characterized by large tracheid

195 lumens, thicker cell walls and high ray tracheid frequency. All of these anatomical features are

196 suggested to facilitate efficient water transport and stem water storage (Martin *et al.*, 2010), but

197 potentially lead to reduced protection from embolism spread between tracheids (pit area

198 hypothesis). In addition, the simple notion that conduit diameter relates to embolism formation

199 is further complicated by the fundamental difference between gymnosperms and angiosperms.

200 Gymnosperms have unicellular tracheids that are a few millimeters long, whereas angiosperms

201 have multicellular vessels with lengths measured in centimeters to meters. This anatomical

202 diversity is most likely responsible for the lack of a simple relationship between conduit diameter

203 and embolism pressure across tracheids and vessels (Sperry *et al.*, 2006).

204

205 These anatomical trade-offs can be considered in light of expected climatic change, although

206 specific predictions are hard to make. In general, drier weather patterns would promote

207 formation of narrower vessels in angiosperm species, a tendency that would limit the probability

208 of embolism formation. This, however, would lead to the reduction of xylem transport capacity,

209 potentially increasing hydraulic resistance and internal tension, thus, eventually leading to zero

210 or negative net effect of this strategy to avoid embolism. Although xylem structure can

211 acclimate to environmental variation during growth and development (Maherali & DeLucia,

212 2000a, Maherali & DeLucia, 2000b, Thomas *et al.,* 2007), the subsequent acclimation of

213 embolism resistance to stress is not possible because xylem conduits are dead at maturity (Choat

214 *et al.*, 2012). Thus, the presence of extreme weather events may further undermine this

215 anatomical adaptation strategy leading to embolism related dieback of already established trees.

216 At the very least, the tendency to grow narrower xylem conduits would most likely result in less

217 efficient transport system and would lead to overall reduction in photosynthetic capacity and

218 reduced growth rates.

219

220 *Wood density*

221

222 Wood density is another morphological parameter often related to a tree's ability to withstand

223 embolism. It depends on cellular wall thicknesses of tracheid and vessels, lumen diameters, and

224 percentage of latewood (part of the wood in a growth ring of a tree that is produced later in the

225 growing season). It was suggested that wood density and specifically wall thickness is related to

226 the risk of cell wall collapse under tension and embolism due to mechanical cell damage

227 (Jacobsen *et al.*, 2005, Sperry *et al.*, 2003, Wagner *et al.*, 1998). Indeed, species characterized

228 by a high wood density and a high thickness-to-span ratio [thickness:span represents the ratio of

229 conduit double wall thickness to lumen diameter, (Pittermann *et al.*, 2006)] of water conducting

230 cells are more resistant to embolism formation (Hacke *et al.*, 2001) and trees producing low-

231 density wood under favorable water availability are more stressed by sudden drought (Rosner *et*

232 *al.*, 2014).

233

234 It is very unlikely that cellular walls directly contribute to embolism spread between conduits.

235 Wall forming cellulose fibers are impregnated with lignin, hemicelluloses and pectin limiting

236 potential radius of pores to be smaller than those existing in bordered pit membranes. However,

237   under changing climatic conditions, induction of drought response might lead to changes in wall

238   surface chemistry (Kostiainen *et al.*, 2006). Lignin is a hydrophobic component of the xylem

239   conduits walls. Thus one can speculate that changes in lignin wall content in response to water

240   stress would be responsible for the increase of wall hydrophobicity. Consequently, the surface

241   would be more likely to nest persistent air pockets responsible for 'air seeding', thus limiting the

242   effectiveness of this response as an embolism avoidance strategy.   Similarly, genetic

243   manipulation aiming at decreased lignin xylem content for fuel production might also have

244   unintended consequences, as a reduction in lignin content generally leads to thinner walls and

245   increases the potential of conduit collapse under tension (Donaldson, 2002) and higher

246   vulnerability to embolism (Voelker *et al.*, 2011).   Again, we are left with highly limited

247   knowledge of natural potential for acclimation of trees to changing climate via adaptation of

248   wood structure.

249

250   *Tree mortality - hydraulic failure*

251

252   In the past 20 years an increased frequency of widespread forest mortality due to drought or

253   temperature stress has been observed in many different tree-dominated ecosystems, such as

254   tropical rainforest (Phillips *et al.*, 2009), temperate mountainous forests, Mediterranean forests,

255   and boreal forests (Carnicer *et al.*, 2011, Peng *et al.*, 2011, van Mantgem *et al.*, 2009). Also, in

256   temperate North American forests some mortality events were associated with "global change-

257   type droughts", defined as severe drought coupled with elevated summer temperatures

258   (Breshears *et al.*, 2005, Shaw *et al.*, 2005, Worrall *et al.*, 2010). Therefore, to make predictions

259   of how future climate change scenarios will affect different biomes it is critical to understand the

260 physiological mechanisms responsible for tree mortality. In this context, we have to consider (i)

261 hydraulic failure and (ii) carbon starvation (McDowell *et al.*, 2008, Nikinmaa *et al.*, 2013,

262 Sevanto *et al.*, 2014).

263

264 Overall, evolution of xylem conduits can be described as an increase in transport efficiency at the

265 expense of embolism protection and vice versa. Trees dominate multiple ecosystems that are

266 characterized by high moisture availability. However, despite over 350 million years of

267 evolution, trees have a limited presence in many drier biomes. Woody plants mostly exist as

268 shrubs that deal with stress related injuries by re-growing lost stems. The absence of trees in dry

269 habitats suggests their limited ability to form a transport system that is perfectly protected from

270 embolism under extreme drought conditions. In areas occupied by trees, they may experience

271 'hydraulic failure' when water loss from transpiration is greater than water uptake by roots,

272 creating high negative xylem tension that may result in embolism (Sevanto *et al.*, 2014, Sperry *et*

273 *al.*, 1998).

274

275 The ability of a plant to protect itself from hydraulic failure is often described in terms of its

276 'hydraulic safety margin' (Meinzer *et al.*, 2009). This trait is often analyzed using 'vulnerability

277 curves' that plot how xylem hydraulic conductivity (Kh) declines as xylem pressure becomes

278 more negative. Declines in Kh are typically expressed relative to the maximum sample Kh as the

279 percentage loss of conductivity (PLC). Such curves are typically sigmoidal in shape with low

280 PLC at xylem pressure near zero and high PLC at large negative pressures. Embolism resistance

281 has been widely documented across species and varies greatly (Maherali *et al.*, 2004). There is

282 also evidence that embolism resistance can vary between populations of the same species (Alder

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283   *et al.*, 1996, Mencuccini & Comstock, 1997, Sparks & Black, 1999) or between closely related

284   genotypes (Barnard *et al.*, 2011, Choat *et al.*, 2007, Neufeld *et al.*, 1992, Pita *et al.*, 2003,

285   Sangsing *et al.*, 2004). Usually, comparisons of vulnerability to embolism among species are

286   represented by the xylem pressure at which a 50% loss of conductivity (P50) occurs. The

287   hydraulic safety margin is defined as the difference between naturally occurring minimum xylem

288   pressures and pressures that would cause 50% of hydraulic dysfunction. The naturally occurring

289   minimum xylem pressure is usually related to stomatal activity and pressure at which stomata

290   start to regulate water loss. Once a plant has reached its P50, it is on the steepest part of the

291   vulnerability curve, which means that even a small decrease in xylem pressure will produce a

292   substantial reduction of Kh (Johnson *et al.*, 2012), increasing the risk of runaway embolism

293   (Tyree & Sperry, 1988). Usually, plants with narrow safety margins experience large amounts of

294   embolism in their respective environments and, therefore, show a high risk of hydraulic failure

295   (Choat *et al.*, 2012).

296

297   Differences in hydraulic safety strategies are reported between angiosperms and conifers.

298   Conifers tend to have greater safety margins than angiosperms (Choat *et al.*, 2012, Meinzer *et*

299   *al.*, 2009) and also experience smaller levels of embolism in the stem while being more

300   permissive of embolism formation in distal organs (i.e. leaves and roots), (Johnson *et al.*, 2012).

301   This strategy can be considered as a safety valve to protect the integrity of the stem hydraulic

302   pathway as it is impossible to replace the main stem while branches and roots can be regenerated

303   or potentially refilled (McCulloh *et al.*, 2011). Angiosperms are characterized by narrow or even

304   negative safety margins making them vulnerable to extensive levels of embolism formation

305   across branches and stems. Plants have evolved mechanisms to restore xylem function (i.e.

14

306 embolism recovery) following events of drought (Johnson *et al.*, 2012). Safety margins of 226

307 woody species among angiosperms and gymnosperms sampled from sites with a wide range of

308 mean annual precipitation and temperature were recently compared in a meta-analysis (Choat *et*

309 *al.*, 2012). Of all species analyzed, 70% were considered to operate at narrow safety margins

310 regardless of climate preferences. However, it is important to mention here that this analysis was

311 exclusively done on small branches that are known to be more vulnerable to embolism than large

312 brunches and trunks (McCulloh *et al.*, 2014). Such a large number of species operating with

313 narrow safety margins may suggest that all biomes will be vulnerable to drought-induced decline

314 with the increases of aridity and temperature that have been predicted for many regions with

315 expected global climate change. Moreover, if extreme weather events increase in frequency and

316 severity, then drought-induced forest decline might occur not only in arid regions but also in wet

317 environments since safety margins are independent of mean annual precipitation. Thus, general

318 trends in the 'hydraulic safety margin' of trees suggest that drought-induced decline and

319 mortality at regional and global scales has the potential to occur (Choat *et al.*, 2012). Yet the

320 magnitude of this effect will depend on the ability of different species to recover from xylem

321 conductivity losses and acclimate via stomata.

322

323 Stomata provide dynamic protection from cavitation by limiting the level of negative water

324 potential that a plant experiences (Meinzer *et al.*, 1992, Sperry & Pockman, 1993). Trees adjust

325 their water use and hydraulic safety through the coordination of hydraulic and stomatal

326 regulations (Brodribb & McAdam, 2011, Choat *et al.*, 2012, Sperry, 2000). Such an association

327 between xylem water potential at the onset of xylem cavitation and leaf water potential triggering

328 stomatal closure are well documented (Brodribb & Holbrook, 2003, Brodribb *et al.*, 2003,

329 Cochard *et al.*, 2002, Hubbard *et al.*, 2001, Klein, 2014, Nardini *et al.*, 2001). Without stomatal

330 control excessive embolism formation would eventually lead to 'runaway embolism', in which

331 embolism decreases hydraulic conductance and xylem water potential, leading to a cycle of

332 embolism that precipitously reduces water conducting capacity until it is completely lost (Tyree

333 & Sperry, 1988). The balance between the temporal dynamics of stomatal closure and stem

334 water potential is species-specific and is often linked to environmental properties. Under

335 changing climatic conditions species might experience a shift in the rate of water stress

336 enactment and, as a result, may be unable to track environmental change with stomatal closure.

337 In fact, a sudden increase in vapor pressure deficit may lead to transient 'wrong way' stomatal

338 response i.e. stomatal opening despite sudden drop in leaf water potential (Buckley, 2005,

339 Buckley *et al.*, 2011) and ultimately cause a runaway embolism. However, even when stomata

340 are capable of reducing transpiration rates, effectively protecting xylem from embolism, doing so

341 comes at the cost of reduced photosynthetic activity and overall loss of carbohydrate reserves,

342 which will likely be detrimental to tree health.

343

344 *Tree mortality – carbohydrate starvation*

345

346 Carbon starvation occurs when stomata are closed to prevent water loss and avoid hydraulic

347 failure. During shorter timeframes, species have strategies that account for the imbalance

348 between carbon uptake by photosynthesis and carbon loss due to respiration and growth.

349 However, prolonged periods of stomatal closure might lead to a negative total carbon balance

350 that depletes carbohydrates reserves and ends in tissue-level carbohydrate starvation (McDowell

351 *et al.*, 2008, McDowell *et al.*, 2013). For some temperate angiosperm species exposed to

352 extreme drought conditions hydraulic failure is the first cause of tree mortality (Barigah *et al.*,

353 2013, Urli *et al.*, 2013). In these species the lethal xylem water potential (xylem pressure

354 correspondent to 50% of mortality) occurred when drought had caused > 88-90% loss of stem

355 hydraulic conductivity (Barigah *et al.*, 2013, Urli *et al.*, 2013). Thus, processes involving

356 hydraulic impairments rather than carbohydrate impairments are considered to be more

357 important for predicting the effects of drought on forests (Anderegg *et al.*, 2012). However,

358 carbon and hydraulic properties cannot be considered in isolation as both are interrelated and

359 changed during mortality of aspen during a widespread die-off event (Anderegg *et al.*, 2012).

360 Evidence of carbon starvation–induced tree mortality is scarce, maybe because it can cause trees

361 to die only after long exposure to relatively mild water stress conditions characterized by

362 extended periods without positive net photosynthesis (Adams *et al.*, 2013), presumably due to

363 the reduction of carbon uptake accompanying prolonged stomatal closure (Mitchell *et al.*, 2013,

364 Ogasa *et al.*, 2013).

365

366 Across gymnosperms it was shown that the major cause of tree mortality is related to drought

367 and occurs when drought causes > 50% loss of stem hydraulic conductivity (P50) (Brodribb *et*

368 *al.*, 2010, Brodribb & Cochard, 2009). However, in *Pinus edulis* both hydraulic failure and

369 carbon starvation can be independent causal factors of tree mortality. Hydraulic failure causes

370 relatively fast tree death that is characterized by rapid decline in leaf water potential, high level

371 loss of hydraulic conductivity, and no changes in carbohydrate reserves at death. Carbon

372 starvation resulting from prolonged shading causes slow tree death characterized by no

373 significant change in leaf water potential and small variations in hydraulic conductivity but

374 significant reduction in carbohydrates reserves. Changing climatic conditions may lead to co-

375 occurrence of both processes, water stress and carbohydrate starvation. Thus mortality in trees

376 currently experiencing reduction in carbohydrate availability due to shading would be

377 accelerated by hydraulic failure and additional reduction of photosynthetic capacity due to

378 stomatal closure (Sevanto *et al.*, 2014).

379

380 While carbohydrate starvation under drought might be a problem in current conditions, the

381 carbohydrate balance of trees growing in elevated CO_2 and temperature, is not clear. In general,

382 both CO_2 and temperature alter tree xylem anatomy (Domec *et al.,* 2010, Maherali & DeLucia,

383 2000a) and wood hydraulic properties (Phillips *et al.,* 2011) such that observed anatomical and

384 physiological changes result in lower drought tolerance (Way *et al.,* 2013). Deciduous boreal

385 trees, for example, showed a greater vulnerability to embolism in leaves exposed to elevated

386 temperature compared to those grown at ambient temperatures, even though vulnerability in

387 stems did not change between treatments (Way *et al.,* 2013). Eucalypt seedlings exposed to

388 moderate drought stress and elevated temperatures showed reduced plant carbon accumulation

389 and increased leaf respiration. When the same seedlings were exposed to moderate drought

390 stress and high CO_2 concentrations, they exhibited increased carbon content and dry mass

391 accumulation. These beneficial effects vanished, however, when drought became more severe

392 (Duan *et al.,* 2013). Unfortunately, only a few studies have analyzed how plants respond to

393 elevated CO_2 concentration and temperature combined (Duan *et al.,* 2013, Maherali & DeLucia,

394 2000a, Phillips *et al.,* 2011) and more information is needed to understand how trees will

395 respond to drought under global change conditions.

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18

Recovery from embolism

Plants have evolved several strategies to prevent and/or mitigate the effects of hydraulic failure due to embolism and to restore xylem transport capacity once embolism occurs (Secchi & Zwieniecki, 2012, Stiller & Sperry, 2002). Restoration of xylem capacity can be divided into two sets of strategies: (1) growth and (2) maintenance. The first group of strategies includes passive, often long-term, responses like shedding leaves or small branches followed by the growth of new shoots (shrubs). They might also include growth of new xylem and the formation of new conduits to assure continuity and capacity of the transport system under adverse conditions (Sperry *et al.*, 1987, Stiller & Sperry, 2002). The second group includes active and often relatively fast physiological responses that result in the restoration of hydraulic capacity by generating positive root pressure (often only found in small herbaceous plants) (Cochard *et al.*, 1994, Ewers *et al.*, 1997, Yang *et al.*, 2012), by stem parenchyma cell activity that results in localized embolism removal in woody plants (Brodersen & McElrone, 2013, Nardini *et al.*, 2011, Salleo *et al.*, 2004), and by strategies that require access to external water (rain, fog or snow) via leaves or buds in order to soak the xylem. The last process has been observed primarily in coniferous species (Laur & Hacke, 2014, Mayr *et al.*, 2014).

The impact of climate change on the first group of strategies would be related to changes in plant growth and would depend on relative changes in carbon fixation and respiration rates and acclimation pattern observed for specific species. Specifically, it is expected that small increases in carbon assimilation due to higher CO_2 concentrations might be offset by increased respiration due to rising average temperatures, suggesting that carbohydrates available for growth

421 (construction) might not change or could even decrease. Thus, the potential to restore hydraulic

422 capacity by conduit replacement, or growing new branches might not keep up with expected losses

423 due to increased rates of embolism formation, effectively leading to increased stress experienced

424 by plants, along with a reduction in gas exchange. Lower gas exchange would further impact a

425 plant's ability to maintain an adequate pool of carbohydrates to replace lost conduits and branches,

426 effectively creating a feed-forward loop that would result in progressive loss of tree vitality,

427 growth rate and a potential yield reduction, as a higher fraction of available resources will be used

428 for construction and maintenance. As the recovery strategies from this first group depend on plant

429 growth and are slow, the effects of climate change in plants relying on these strategies would be

430 expected to occur over extended periods of time (years). The effects of climate change will most

431 likely be reflected in reduced tree crown size due to die-back of branches and in reduced total leaf

432 area resulting in less overall transpiration. This reduction in crown size would match transport

433 capacity of the stem but it would reduce tree vitality and might be related to increased mortality.

434 Although, studies of the long term effect of increased temperature on respiration suggest that some

435 plants can acclimate and respiration can be greatly reduced in higher temperatures (Atkin *et al.,*

436 2005, Atkin & Tjoelker, 2003). This may still lead to higher respiration rates in warmer

437 environments than in cooler ones, but not to the degree implied by short-term temperature

438 changes.

439

440

441 The second group of strategies that might allow trees to recover from xylem hydraulic capacity

442 losses depends on the use of energy to dynamically repair embolized conduits under the presence

443 of adverse conditions, like tension or transpiration. Such strategies potentially allow plants greater

444 flexibility in response to periods of drought and mediate temporal losses to photosynthetic

445 capacity. Reconciliation of embolism recovery with continued xylem tension has proven to be

446 difficult to understand (Holbrook & Zwieniecki, 1999, Tyree *et al.*, 1999), and only recently has *in*

447 *vivo* imaging provided strong support for the notion that plants can recover embolized vessels,

448 even under low or moderate stress levels (Brodersen *et al.*, 2010, Clearwater & Goldstein, 2005,

449 Zwieniecki *et al.*, 2013). However, despite significant scientific efforts (Nardini *et al.*, 2011,

450 Salleo *et al.*, 1996, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011, Zwieniecki &

451 Holbrook, 2009), the mechanisms responsible for embolism recovery under negative pressure is

452 still not well understood.

453 Predicting future climate impact on the embolism recovery process is difficult because the specific

454 physiological mechanisms involved are still unknown, however, a more general look at the impact

455 of climate change on embolism recovery is possible.

456

457 While embolism formation is a spontaneous, purely physical process related to the degree

458 of tension in the water column and to the physicochemical and anatomical properties of the wood

459 (Brenner, 1995, Tyree & Zimmermann, 2002), embolism removal requires that empty vessels fill

460 with water against existing energy gradients. Thus, recovery from embolism cannot happen

461 spontaneously and requires physiological activity that promotes water flow into embolized vessels

462 (Holbrook & Zwieniecki, 1999, Salleo *et al.*, 2004, Secchi *et al.*, 2011, Tyree *et al.*, 1999,

463 Zwieniecki & Holbrook, 2009). Visual evidence from cryo-SEM studies, magnetic resonance

464 imagining (MRI) observations, and computed tomography (CT)-scans showed that water (xylem

465 sap) can return to empty vessels, suggesting that plants have the ability to restore functionality in

466 the xylem (Clearwater & Goldstein, 2005, Holbrook *et al.*, 2001, Scheenen *et al.*, 2007). A more

467   recent study showed that in grapevine vessels, where the bulk xylem tissue was still under tension,

468   water droplets preferentially formed on the vessel walls adjacent to parenchyma cells and that

469   these droplets grow until the lumen was completely refilled (Brodersen *et al.*, 2010), although it

470   was not proven that these refilled lumens returned to full function i.e. being able to transport water

471   under tension.   Droplet formation on the walls of empty vessels that are in contact with

472   parenchyma cells support predictions that these living cells play a critical role in embolism

473   removal by supplying energy, and possibly water, to drive the restoration of xylem hydraulic

474   function (Brodersen & McElrone, 2013). If xylem parenchyma cells supply a significant fraction

475   of water required for filling embolized vessels, water must pass a cellular membrane and, thus, the

476   flow must be facilitated by aquaporins (membrane proteins that facilitate water transport across

477   the cellular membrane). Studies on walnut (*Juglans regia*) showed that higher expression of two

478   aquaporin proteins (JrPIP2.1 and JrPIP2.2) was observed in vessel-associated parenchyma cells at

479   the same time that recovery from embolism took place (Sakr *et al.*, 2003). Moreover, expression

480   levels of several PIP1 and PIP2 genes were shown to increase during the recovery process in two

481   other species *Populus trichocarpa* and *Vitis vinifera* (Kaldenhoff *et al.*, 2008, Perrone *et al.*, 2012,

482   Secchi *et al.*, 2011, Secchi & Zwieniecki, 2010). Detailed analysis of how the transcriptome

483   responds to the presence of embolism in *P. trichocarpa* and *V. vinifera* petioles revealed that

484   several aquaporin subfamilies were strongly up-regulated during refilling (Perrone *et al.*, 2012,

485   Secchi *et al.*, 2011) especially PIP1, PIP2, and TIP.

486

487   Recovery from embolism requires a source of water to fill the empty vessels and a source of

488   energy to overcome existing energy gradients acting against water flow. Living parenchyma cells

489   can, parsimoniously, provide both. This assumption is supported by the inhibition of recovery in

490 response to either physical damage to phloem or metabolic inhibition of living cells in stems

491 (Bucci *et al.*, 2003, Salleo *et al.*, 2004, Zwieniecki *et al.*, 2004). A series of models have proposed

492 that sugars needed as osmoticum for refilling can be unloaded into the embolized vessels from ray

493 parenchyma cells. Sugars can either be transported from the phloem or be released from starch

494 stored in the stem. Once in the apoplast they can create an osmotic gradient that drives water from

495 the parenchyma to the empty vessels (Nardini *et al.*, 2011, Secchi & Zwieniecki, 2011, Zwieniecki

496 & Holbrook, 2009). For this process to work, the presence of embolism should alter carbohydrate

497 metabolism and carbon partitioning between starch and soluble sugars in the xylem parenchyma

498 and potentially influence both enzyme activities and gene expression. Indeed, both visualization

499 techniques and enzymatic analysis of non-structural carbohydrates levels in xylem of *L. nobilis*

500 and *P. trichocarpa* experiencing high levels of embolism demonstrated that starch content in stem

501 parenchyma cells decreased and that soluble sugar levels increased (Nardini *et al.*, 2011, Regier *et*

502 *al.*, 2009, Salleo *et al.*, 2009, Secchi & Zwieniecki, 2011). Furthermore, a drop in starch content

503 was associated with changes in gene expression, especially down-regulation of the

504 monosaccharide metabolic pathway (including enzymes involved in starch synthesis) and strong

505 up-regulation of the disaccharide metabolic pathway that also includes starch degradation enzymes

506 like alpha- and beta-amylases in the stems of *P. trichocarpa* (Secchi & Zwieniecki, 2011). The

507 same study also revealed an up-regulation of genes from ion transport and carbon metabolism

508 ontology groups. Similarly, an up-regulation of carbon metabolism was exhibited during petiole

509 recovery in grapevine (Perrone *et al.*, 2012). This transcription level response matched results

510 obtained from chemical analysis of liquid collected from non-functional (embolized) vessels,

511 where elevated levels of sugars and ions were found. However, the total osmotic concentration

512 increase was relatively small and could only account for recovery under low tension levels (Secchi

513 & Zwieniecki, 2012), thus, a significant relief from stress is most likely required before functional

514 recovery from embolism and restoration of hydraulic activity can occur.

515

516 A recent evaluation of the role of aquaporins in recovery of trees from embolism using transgenic

517 plants with down-regulated expression of the PIP1 subfamily (Secchi & Zwieniecki, 2014) in

518 combination with visual observation of the recovery process (Brodersen *et al.*, 2010, Zwieniecki *et*

519 *al.*, 2013) may suggest a new view of the embolism-recovery cycle. It is possible that embolism

520 level is a net effect of embolism formation (with a rate that is positively related to stress level) and

521 recovery (refilling, with a rate that is inversely related to stress level). This notion would explain

522 the observed higher vulnerability to embolism in the transformed plants even though the plants

523 show no anatomical changes that might affect embolism rate (Secchi & Zwieniecki, 2014): the

524 shift in vulnerability would be achieved not by any change in the rate of embolism initiation, but

525 rather by a differential rate of embolism recovery due to a lower membrane permeability of xylem

526 parenchyma cells. This view of the embolism-recovery cycle might suggest that a vulnerability

527 curve estimated from fresh material collected in the field reflects the current balance between

528 embolism and recovery processes, rather than a true measure of xylem embolism vulnerability.

529

530 Identifying how xylem recovers after embolism formation is fundamental to understanding the

531 impact of climate change on water transport and tree survival. As described above, xylem

532 recovery is highly variable among tree species and even among individual plants of the same

533 species, as recovery might depend on the current level of embolism and health status of the plant

534 (Hacke & Sperry, 2003, Holbrook *et al.*, 2001). For example, angiosperms are shown to recover

535 from embolism within days or even hours if provided relief from stress (Bucci *et al.*, 2003, Ogasa

536 *et al.*, 2013, Salleo *et al.*, 2004) while conifers tend to show very slow recovery (days or even

537 months). Such slow recovery would suggest that embolized tracheids either are refilling very

538 slowly or must be replaced by new tracheid in order to restore xylem hydraulic capacity (Brodribb

539 *et al.*, 2010, Brodribb & Cochard, 2009). However, some evidence of the fast recovery process in

540 gymnosperms has been recently shown in Norway spruce trees (*Picea abies*) that were able to

541 recover embolized xylem in later winter using melting snow as the source of water (Mayr *et al.*,

542 2014). This process was shown to be related to starch metabolism and an increase of aquaporin

543 abundance. Similarly, the role of aquaporins in facilitating radial water movement from the needle

544 epidermis towards the vascular tissue was also found in *Picea glauca* suggesting that if exposed to

545 reduced stress and foliar availability of water even conifers may have evolved a physiological

546 strategy to increase rate of recovery from embolism with refilling occuring in a matter of hours

547 (Laur & Hacke, 2014). When compared with angiosperms, gymnosperms tend to have less

548 parenchyma cells in the xylem and lower non-structural carbohydrate abundance in their wood.

549 As the ability to rapidly remove embolism relies on the proximity of parenchyma cells to xylem

550 conduits as a source of energy and water, this might explain the need for larger safety margin in

551 conifers relative to angiosperms at comparable levels of embolism (Johnson *et al.*, 2012). The

552 role of xylem parenchyma in embolism recovery is further underlined by a study showing that

553 among 47 plant species, including angiosperms and gymnosperms, axial parenchyma was

554 commonly present in most woody angiosperm species able to recover from embolism formation.

555 The few cases where parenchyma was absent were mostly in herbaceous annual plants without

556 secondary xylem where the recovery process could only be accounted by the presence of root

557 pressure (Brodersen & McElrone, 2013).

558

Xylem function in the 'new climate normal'

We are just beginning to understand fully the complexity of water transport function in the xylem--including its structure, rheology, chemistry, and physiology. Our knowledge still has many gaps and only a superficial knowledge exists regarding both embolism formation and the process of recovery from embolism in woody plant xylem. Yet, we face the prospect of rapid changes to climatic conditions that are shorter than the lifespan of a single tree. Orchards and forests growing today will experience a transition period to the 'new climate normal' while we have very few tools to predict climate impact on tree growth, yield and survival, and even less knowledge to inform breeding efforts aimed at mediating the negative impact of changing climate. However, by scrutinizing available knowledge we can filter out a few important aspects of how climate change may impact xylem function and try to predict the most likely scenarios describing the effect of increased temperature, reduced water availability, and increased frequency and duration of extreme weather events on tree hydraulic system capacity.

We can assume that reduced water availability due to a drop in precipitation abundance, frequency, or both, would lead to extended periods of time during which trees would experience xylem tension levels near their 'safety margin', i.e. the level of tension when stomatal conductance is reduced to protect xylem from excessive embolism formation (Figure 2). "Safety margin" is an evolved trait that likely is a deeply embedded property of species-specific survival strategies and is not easily changed. Thus, prolonged periods of drought that force stomata to close should result in loss of photosynthetic capacity and a reduction in non-structural carbohydrate availability needed by a tree to maintain transport capacity under drought and/or

582 restore it during periods of stress recovery following the relief provided by a rainfall or fog event

583 (Figure 2).   Thus, under drought conditions, trees might experience an accumulation of

584 embolized conduits in stems over time, leading to reduced benefits from their evolved 'safety

585 margins'.   We can expect that in the near future the potential for 'run-away cavitation' will

586 increase, especially in species operating at or near their physiological thresholds and not having a

587 strong stomatal control.   In addition, if embolism level in a plant reflects the balance between

588 embolism formation rate and the capacity to refill embolized vessels using available energy, then

589 prolonged periods of drought would shift that balance toward trees capable of withstanding

590 higher levels of embolism.   Under low water potential, the rate of new embolism formation will

591 exceed the rate of recovery. As frequency and length of stress relief periods are expected to

592 diminish, the recovery process will be shortened as well, forcing trees to operate at continuously

593 higher level of embolism.   Operating at higher level of embolism will reduce the capacity of

594 trees to photosynthesize, as higher stress levels will force stomatal closure due to higher stem

595 hydraulic resistance.   This general reduction in carbon assimilation will reduce the total

596 carbohydrate pool that could affect all aspects of plant growth, protection, and yield.   This

597 reduction in production will be magnified by rising average temperatures that would increase

598 plant respiration which will further decrease the size of the total carbohydrate pool and the

599 ability of trees to maintain xylem transport capacity.   Such significant reductions in the stem

600 non-structural carbohydrate pool would also lead to reduced growth, higher rates of mortality,

601 and may reduce the yield of commodity trees.

602 In conclusion, mediating future climate impacts and guiding breeding programs will require

603 better knowledge of tree stress physiology.   Specifically, we should invest in improving our

604 understanding of xylem physiology under variable levels of stress.   We are missing basic

27

605   information regarding daily carbohydrate balance, structure of carbohydrate use, and plant

606   flexibility to shift energy among multiple competing demands like maintenance, growth, stress

607   response, and recovery reserves. We are also missing fundamental knowledge regarding the

608   biological significance of how observed embolism/recovery relates to basic phenomena at both

609   cellular and whole plant levels. Without this basic understanding, we cannot confidently predict

610   how trees will respond to the 'new climate normal'.

611

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615

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Figure legends

Figure 1

Embolism formation in a vessel may originate from multiple sources including (1) breaking of

the air/water interface at the bordered pit membrane if an adjacent vessel was already embolized,

(2) expansion of air pockets from vessel crevices or (3) expansion of persistent air bubbles on the

wall surface that could be remnants of previous embolism. The air-water interface might be in

equilibrium if its surface tension is sufficient to oppose the tension from water in the

transpiration stream. If tension from water in the transpiration stream exceeds water surface

tension the bubble will expand. Such an expanding bubble will continuously decrease its radius

if water/wall contact is fixed in one position. If tension in the vessel exceeds the maximum

tension sustained by the bubble (at critical radius), the air-water interface breaks and the bubble

expands forming an embolism.

Figure 2

Figure 2

Xylem transport capacity depends on the number of functional vessels (I) that can transport

water under tension. The number of functional vessels is the total number of vessels in the stem

minus embolized vessels and those refilled but not yet functionally linked to vessels conducting

water under tension. Rates of embolism formation (II) and refilling (III) determine the number

of non-functional vessels while the rate of returning vessels to functional state (IV) determine

number of vessel capable of sustaining tension (V). These rates are under physiological control

that will be impacted by climate change. Specifically, increasing atmospheric vapor pressure

1026   deficit (VPD) may result in increasing transpiration rates (a) that in turn would increase water

1027   tension in the xylem (b). Increasing xylem water tension would increase the rate of embolism

1028   formation (c) and effectively the number of embolized vessels. High tension would also

1029   negatively impact both potential for refilling of vessels (d) and rate of returning vessels to a

1030   functional state (e). In addition, climatic changes are expected to affect solar radiation and

1031   temperature which would have a major impact on both photosynthesis and respiration rates (f).

1032   Respiration rates are expected to increase with increasing temperature while photosynthesis rates

1033   are expected decrease due to both higher temperature and stomatal closure resulting from higher

1034   tension levels (g).   Together such an impact would reduce the level of non-structural

1035   carbohydrates stored in the stem that would be available to provide energy required for vessel

1036   refilling (h), thus reducing the rate of refilling (i) and returning vessels to a functional state (k).
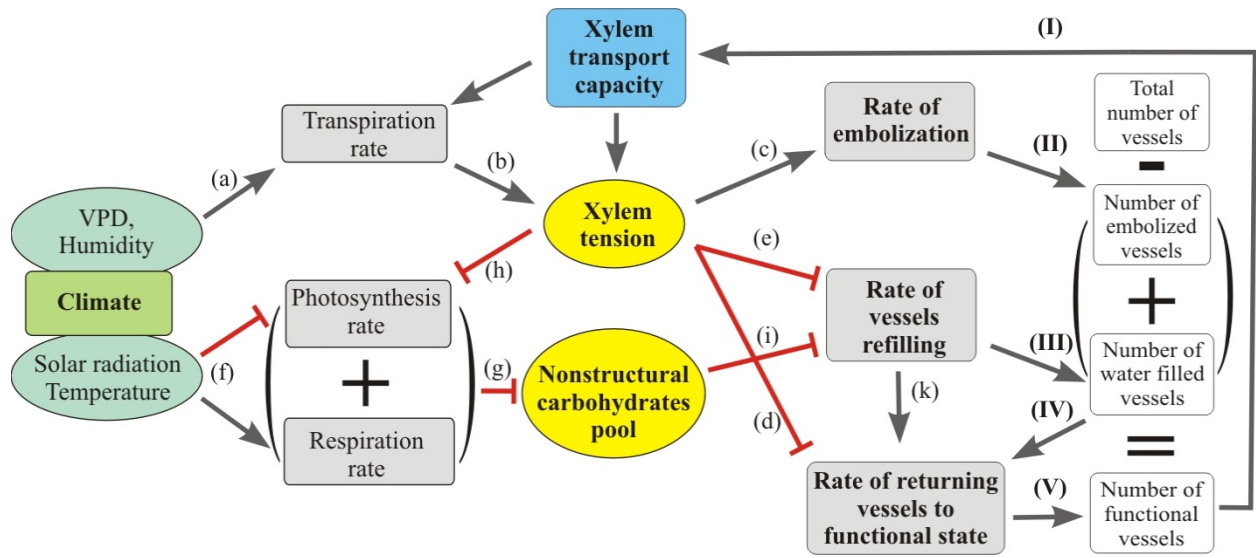
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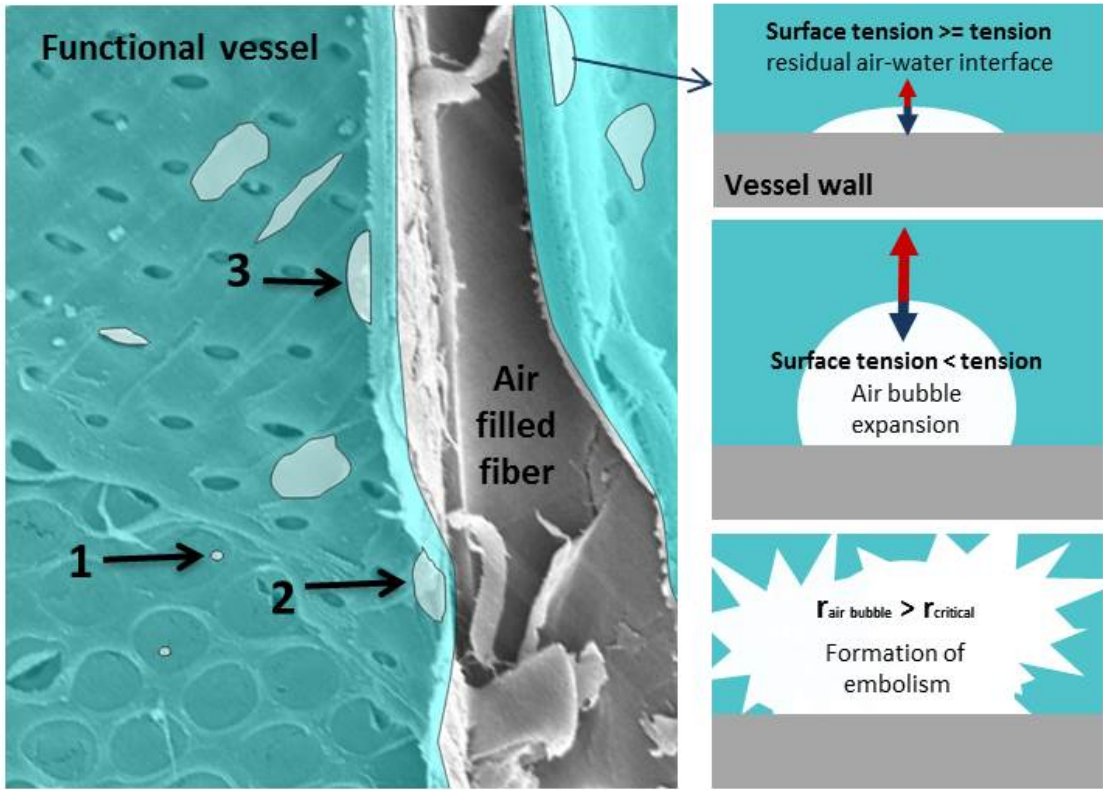
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