Running Head:

The limits of drought tolerance

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Research Area:

Ecophysiology and Sustainability
Title: Extreme aridity pushes trees to their physical limits

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One-sentence summary:

An Australian desert tree species displays record drought-resistance, bringing it remarkably close to the physical limit for liquid water-transport in vascular plants.
Footnotes:

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Drought-induced hydraulic failure is a leading cause of mortality of trees (McDowell et al., 2008; Anderegg et al., 2012), and has become a major concern in light of future climate predictions, with forests across the world showing signs of vulnerability to intense and prolonged drought events (Allen et al., 2010). We show here that Callitris tuberculata, a conifer species from extremely dry areas of Western Australia, is the most cavitation-resistant tree species in the world to date (mean $P_{50} = -18.8$ MPa). Hydraulic conductance is maintained in these plants at pressures remarkably close to the practical limit of water metastability, suggesting that liquid water transport under the Cohesion-Tension theory has reached its operational boundary.

Coping with desiccation is one of the greatest challenges faced by plant life on land and the evolution of specialized tissue for the transport of water played a key role in the colonization of continents (Black and Pritchard, 2002; Sperry, 2003). As well as evolving mechanisms to reduce water-loss (e.g. stomata and waxy leaf surfaces), land plants also have to provide their aerial organs with water to fuel photosynthesis, supply metabolism and control leaf temperature through transpiration, even when water availability is low. Driven by competition for limited resources, plants have perfected their vascular systems over hundreds of millions of years, enabling vertical transport of water to heights in excess of 100 m above the ground and resulting in the dominance of trees across a wide range of terrestrial ecosystems. However, trees are excluded from extremely dry and cold climates; we propose here that an absolute limit for water transport in trees exists, set by the physical properties of liquid water, restricting the existence of woody non-succulent trees in dry deserts.

Movement of water against gravity in trees is driven by a remarkably simple process, described by the tension-cohesion theory (Tyree and Sperry, 1989; Tyree, 1997; Sperry, 2003). Evaporation at the leaf surface causes water in the plant to move up through a specialized transport tissue and drives water uptake from the soil. This leads water in the xylem to be stretched at negative pressures. However, cohesive forces due to hydrogen bonds bind water molecules together and also onto cell walls, sufficiently to maintain water in a liquid, yet metastable state prone to sudden vaporization by cavitation. In moist soils these pressures are moderate, typically above -2 MPa, but during drought, they decrease considerably as plants are forced to extract water from drying soil, which reduces the stability of the water column. Below a specific pressure threshold, cavitation events occur when air-water menisci located at pores between xylem conduits break (Tyree, 1997; Cochard et al.,
5, 2009; Mayr et al., 2014), vaporizing sap, reducing xylem conductance and eventually leading to plant death by desiccation (Brodribb et al., 2010; Urli et al., 2013). The xylem pressure at which cavitation occurs ($P_{50}$ – leading to 50% loss of hydraulic function) is a trait that varies widely across species (Delzon et al., 2010; Bouche et al., 2014), and links with climate, i.e.
xeric species are more resistant to cavitation than species that occupy more mesic habitats (Brodribb and Hill, 1999; Maherali et al., 2004; Choat et al., 2012; Pittermann et al., 2012). Conifers are generally more resistant to cavitation than angiosperms (Maherali et al., 2004; Choat et al., 2012), likely due to the presence of a pressure-activated safety valve that reduces the spread of air between xylem cells (Bouche et al., 2014). The most cavitation resistant trees known to date are conifers of the genus Callitris from Australia and New-Caledonia, with in particular C. columellaris displaying a $P_{50}$ of -16 MPa (Brodribb et al., 2010). Here, we present the record cavitation resistance of Callitris tuberculata (R.Br. ex R.T. Baker & H.G. Smith. – Cupressaceae; Fig. 1). This tree species is common in extremely arid ecosystems of South-western Australia where its range stretches far into the Great Victoria Desert (Fig. 1B). In this area, the climate is dry and hot, with most rainfall occurring during a short wet season (Supplemental Fig. S1). At its most extreme margin the average annual rainfall is below 180 mm, and annual evapotranspiration can exceed rainfall 10-times, presumably making this species one of the most drought-tolerant trees in the world.

Our results show that this species from the desert tree line produces a previously unparalleled $P_{50}$ of $-18.8 \pm 0.6$ MPa (SE; n=9; Fig. 2 and Supplemental Fig. S2, Supplemental Table S1), making it the most drought resistant tree ever measured. At -21 MPa, the lowest pressure we could achieve with our device (thus extending the experimental xylem pressure range by nearly 20%, from -17.3 MPa (Brodribb et al., 2010)), around 25-50% of xylem conduits were still functional, with the final cavitation events predicted to occur at about -25 MPa. Could evolution push xylem pressure to more extreme values to enable this species to colonize even drier habitats? Strikingly, physics’ answer is no, as C. tuberculata has reached the operational limit of water metastability.

According to the Classical Nucleation Theory (Debenedetti, 1996), homogeneous water cavitation occurs at pressures below -120 MPa at ambient temperature, as has been verified by experiments using microscopic liquid water inclusions in quartz (Zheng et al., 1991; Azouzi et al., 2013). Conversely, other experimental data suggest that bulk cavitation occurs heterogeneously on ubiquitous impurities at much higher pressures, from -20 to -30 MPa depending on the method used to induce negative pressure, water purity and water temperature (see review in Caupin et al., 2012). Consistently, in recent experiments based on a “synthetic tree” (Wheeler and Stroock, 2008; Vincent et al., 2012) bulk cavitation rapidly occurs at tensions of around -22 MPa. The presence of impurities, dissolved minerals and nutrients in xylem sap (Buhtz et al., 2004; Krishnan et al., 2011) leads us to speculate that
bulk cavitation in tree xylem will likely occur at similar pressures, setting an absolute physical limit for water transport in trees during drought.

Drought stress has pushed this species to evolve the most resistant xylem, to the point that *Callitris tuberculata* is able to potentially maintain water flow up to the limit of physical
properties of liquid water. Already growing in an environment severely limited by water-availability, its adaptation to future conditions (i.e. by lowering $P_{50}$) may be restricted by hitting this physical boundary. Indeed, in South-western Australia, a strong trend towards a drier and warmer climate has been evidenced over the 20th century (see insert in Fig. 1B). This could lead to a contraction of forest at the desert margin, but also offers the possibility of a range expansion into historically wetter regions to the South-West.

Hydraulic failure is one of the main hypotheses to explain tree death following prolonged periods of drought (Anderegg et al., 2012). Experiments have shown that after reaching cavitation levels of around 50% in conifers or 90% in Angiosperms, trees cannot recover (Brodribb et al., 2010; Urli et al., 2013). Furthermore, a recent study showed that trees in all ecosystems function with similarly narrow safety margins regarding this threshold to cavitation (Choat et al., 2012). Like South-western Australia, many regions are projected to suffer from increasingly frequent and severe droughts (Stocker et al., 2013), highlighting the need to better understand the physiological responses of trees to drought stress to improve predictions of the impact of climate change on forests and woodlands. Our results suggest that *Callitris tuberculata* is an ideal candidate for further investigations, for example into xylem anatomy modifications allowing evolution of increased cavitation resistance. Evolution towards xylem safety from cavitation leads to a tolerance strategy, allowing plants to maintain limited function even in stressed conditions. We have discovered an absolute limit to this evolutionary path due to water metastability in the xylem, explaining why trees tend to be excluded from the most arid ecosystems.


**Supplemental material:**

**Materials and Methods:**

**Sampling**

As part of investigations into record cavitation-resistant conifers, we collected samples (branches of about 40 cm) from a population of *Callitris tuberculata* near Lake Grace (WA, Australia) in July 2014. They were immediately wrapped in wet paper towels with foliage removed, and sent for measurement at the BIOGECO lab at INRA - University of Bordeaux (France; http://sylvain-delzon.com/?page_id=536) where they were kept in dark, damp and refrigerated conditions until measurements were conducted.

**Vulnerability curves**

Cavitation resistance was estimated with the flow-centrifuge technique, based on the Cavitron method (Cochard *et al.*, 2005), in which centrifugation is used to induce negative pressure in the xylem of the sample and conductance is monitored during spinning (as measured by the water flow through the stem). Samples were cut to length (27 cm), then bark was removed and the ends were re-cut with a clean razor blade. We used a specially designed centrifuge rotor, reinforced to allow previously unattainable speeds of above 13000 rpm. We thus reached a record maximum speed of 15000 rpm, which induces xylem pressure of around -21 MPa. Maximum conductance is estimated at low speed (high pressure), then we gradually increase rotation speed, repeatedly measuring conductance at least 3 times at each pressure step. As xylem pressure is forced to more negative values, cavitation events occur, leading to a drop in hydraulic conductance, which is classically represented as so-called vulnerability curves, percent loss of conductance (or PLC) as a function of pressure. Each individual vulnerability curve was fit using the Pammenter model (Pammenter and Vander Willigen, 1998), with $P_{50}$ derived as the pressure leading to a 50% decrease in conductance (Supplemental Fig. S2). This procedure has been extensively described elsewhere, see for example Methods in Delzon et al. (2010) and Jansen et al. (2012). Individual $P_{50}$ and other parameters for each individual are presented in Supplemental Table S1. Conductance measurements for all individuals were pooled and binned into 15 pressure classes, to create an average vulnerability curve (Fig. 1). The shaded area in Fig. 1 represents the 95% confidence interval for the mean obtained with the CLM option in the Means Procedure in SAS. All statistical analyses was conducted using SAS software (SAS 9.4 Institute, Cary, NC, USA).
This method also enables estimation of xylem specific conductivity, a measure of xylem capacity to transport water. No significant correlation with xylem safety from cavitation was found within this population of *C. tuberculata* (regression analysis $r^2=0.035$; $P=0.63$), indicating absence of a functional trade-off between safety and efficiency.

**Cavitation resistance data**

We extracted published measurements of cavitation resistance from Choat et al. (2012) and Bouche et al. (2014) of both Angiosperm and Gymnosperm species to allow comparison of *Callitris tuberculata* to other species. These datasets are available online respectively from the [Nature](http://www.nature.com) and [Journal of Experimental Botany](http://www.jeb.org) websites.

**Bulk cavitation curves**

Bulk heterogeneous cavitation curves for temperatures of 20, 30 and 40°C (likely to occur in the xylem of *C. tuberculata* during summer drought) were constructed based on equation 12 in Herbert et al. (2006).

**Climate data**

Annual temperature and precipitation deviation from the 1961-1990 average was obtained from the Australian Bureau of Meteorology ([http://www.bom.gov.au](http://www.bom.gov.au)). *Callitris tuberculata* occurrences were downloaded from the Global Biodiversity Information Facility (GBIF; [http://www.gbif.org](http://www.gbif.org)), and used to outline an approximate distribution range. Climate layers were then obtained from Worldclim ([Hijmans et al.](http://www.worldclim.org), 2005) and the Global Aridity Index and PET datasets ([http://www.cgiar-csi.org/data/global-aridity-and-pet-database](http://www.cgiar-csi.org/data/global-aridity-and-pet-database)), and data for each location was extracted in QGIS 2.4.0 (Quantum, 2011) using the point-sampling tool. We show here the average monthly precipitations and maximum daily temperature for all locations obtained from GBIF (Supplemental Fig. S1).
Literature cited:


Figure S1. Climate diagram of average monthly precipitations and maximum temperatures for *Callitris tuberculata*’s estimated range. Black bars represent monthly precipitations, and the red curve shows maximum temperatures.

Figure S2. Vulnerability curves for nine individual trees of *Callitris tuberculata*. Each point represents mean value of percent loss of hydraulic conductance over at least three measurements of hydraulic conductance (bars represent ± standard error). Lines represent the Pammenter model fit to each individual curve, and dashed lines show where the model was expanded beyond experimental data.

Table S1. Cavitation resistance parameters for each individual. $P_{50}$, $P_{12}$, and $P_{88}$ are, respectively, the xylem pressure inducing 50%, 12% and 88% loss of xylem hydraulic conductance. $S$ is the slope of the vulnerability curve at $P_{50}$. $k_s$ is the specific xylem conductivity, a standardized measurement of the samples’ capacity to transport water.
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<th>Individual</th>
<th>$P_{50}$ (MPa)</th>
<th>$P_{12}$ (MPa)</th>
<th>$P_{88}$ (MPa)</th>
<th>$S$ (% MPa$^{-1}$)</th>
<th>$k_s$ (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$)</th>
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