

## LETTER

# Plant resistance to drought depends on timely stomatal closure

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### Abstract

Stomata play a significant role in the Earth's water and carbon cycles, by regulating gaseous exchanges between the plant and the atmosphere. Under drought conditions, stomatal control of transpiration has long been thought to be closely coordinated with the decrease in hydraulic capacity (hydraulic failure due to xylem embolism). We tested this hypothesis by coupling a meta-analysis of functional traits related to the stomatal response to drought and embolism resistance with simulations from a soil–plant hydraulic model. We report here a previously unreported phenomenon: the existence of an absolute limit by which stomata closure must occur to avoid rapid death in drought conditions. The water potential causing stomatal closure and the xylem pressure at the onset of embolism formation were equal for only a small number of species, and the difference between these two traits (i.e. safety margins) increased continuously with increasing embolism resistance. Our findings demonstrate the need to revise current views about the functional coordination between stomata and hydraulic traits and provide a mechanistic framework for modeling plant mortality under drought conditions.

### Keywords

Dieback, drought, stomata, tree mortality, xylem embolism.

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## INTRODUCTION

Recent drought episodes have been identified as the triggers for widespread plant mortality events around the world (Allen *et al.* 2010; Carnicer *et al.* 2011; Park Williams *et al.* 2012). They have had huge consequences for the productivity of the land (Ciais *et al.* 2005) and have undoubtedly affected a panel of ecosystem services (Anderegg *et al.* 2012). Identifying the mechanisms and traits underlying drought resistance will be essential if we have to understand and predict the impact of widespread droughts over many land areas. Experimental studies have provided empirical evidence that failure of the water transport system is tightly linked to tree desiccation and mortality in drought conditions. Support for this hypothesis was recently provided by a study reporting that hydraulic traits explain cross-species patterns of drought-induced mortality at the global scale (Anderegg *et al.* 2016). Two types of traits are thought to be involved in plant hydraulic failure under drought conditions: hydraulic traits ensuring the integrity of the hydraulic system under water deficit (Choat *et al.* 2012), and stomatal traits controlling gas exchange at the leaf surface (Klein 2014). However, efforts to model tree mortality in response to drought are hindered by a lack of understanding of how and why these traits covary at the global scale, and interact to define physiological dysfunctions under drought stress (McDowell *et al.* 2013). In this study, we analysed the overall connections between these two types of traits for the full range of drought resistance, using a soil–plant hydraulic model, and we provide a new formal framework for predicting plant mortality under drought.

The stomata have two key functions: controlling transpiration, which supplies nutrients and regulates leaf temperature, and controlling the entry of CO<sub>2</sub> into the leaf. Stomatal closure

in response to water deficit is the primary limitation to photosynthesis (Flexas & Medrano 2002), and constitutes a key cost in terms of plant growth and temperature regulation under drought conditions. However, stomatal closure also limits excessive decreases in water potential (quantified as a negative pressure,  $\psi$ ) in the plant, thereby ensuring that water demand from the leaves does not exceed the supply capacity of the hydraulic system, which would lead to embolism of the vascular system and complete desiccation of the plant. These key, but opposing roles of stomata in regulating CO<sub>2</sub> influx and H<sub>2</sub>O loss pose a dilemma that has occupied scientists for centuries (Bessey 1898; Darwin 1898) and has led to the view that plant stomata probably operate at the edge of the supply capacity of the plant's hydraulic system, to balance different costs, such as productivity and leaf temperature regulation during drought (Tyree & Sperry 1988; Cruiziat *et al.* 2002; Sperry 2004).

Conversely, maintenance of the supply capacity of the hydraulic system depends on the ability of a species to resist embolism at the highly negative pressure caused by soil water deficit. Embolism resistance is usually quantified as the value of  $\psi$  causing 50% embolism ( $\Psi_{50}$ ), and the rate of embolism spread per unit drop in water potential (*slope*). From these two traits, the  $\Psi$  at the onset of embolism formation can be calculated ( $\Psi_{12}$ , Text S1), providing a more conservative estimate of the functional limit to the hydraulic system. Embolism resistance varies considerably between species and with the dryness of species habitat (Maherali *et al.* 2004; Choat *et al.* 2012; Lens *et al.* 2016; Larter *et al.* 2017). A recent study suggested that hydraulic systems highly resistant to embolism evolved in response to the selective pressure associated with increasing drought levels during a paleoclimatic crisis (Pittermann *et al.* 2012; Larter *et al.* 2017). Some contemporary plants have extremely drought-resistant vascular

systems, with  $\Psi_{50}$  values reaching  $-19$  MPa (Larter *et al.* 2015).

These findings have led to the suggestion that an efficient match between the capacity of the hydraulic system to sustain water deficit (i.e. embolism resistance) and the regulation of demand by the stomata is a prerequisite for the maximisation of gas exchange without desiccation (Tyree & Sperry 1988; Jones & Sutherland 1991; Sperry *et al.* 1998; Sperry 2004). This notion naturally leads to the hypothesis that stomatal behaviour and embolism resistance have followed a similar evolutionary trajectory under drought constraints, and that plants have increased their intrinsic embolism resistance to allow stomata to close later during drought, thereby maximising plant productivity (Cruziat *et al.* 2002; Klein 2014; Skelton *et al.* 2015; Anderegg *et al.* 2016). The coordination of stomatal and hydraulic traits and their role in shaping drought resistance have yet to be addressed on a global scale. Such studies would help to clarify the interplay between mechanisms and plant traits in defining the physiological dysfunctions occurring under drought stress, which remains one of the principal challenges faced in the modelling of tree mortality in response to drought.

In this study, we gathered data for different stomatal regulation traits and embolism resistance traits for more than 100 species from different biomes, to explore their covariation empirically over the full range of drought resistance. We then used a soil–plant water transport model to elucidate how different associations between  $\Psi_{\text{close}}$  and  $\Psi_{50}$  determine the time until hydraulic failure during drought. We validated model predictions, using empirical data for time to shoot death collected in drought mortality experiments (Brodribb & Cochard 2009; Barigah *et al.* 2013; Urli *et al.* 2013; Li *et al.* 2015), to provide a conceptual framework for predicting plant mortality under drought conditions.

## MATERIALS AND METHODS

### Data meta-analysis

Various measurement artefacts are known to have tainted embolism measurements in recent decades (Cochard *et al.* 2013, 2015), and recent direct observations of the xylem content by X-ray tomography have confirmed the need for caution when selecting embolism data (Beikircher *et al.* 2010; Choat *et al.* 2014, 2016; Cochard *et al.* 2015; Torres-Ruiz *et al.* 2015). We chose to use a conservative dataset for this study. We therefore calculated  $\psi_{12}$ ,  $\psi_{50}$  and *slope* from S-shaped vulnerability curves obtained and published by our group over the last 20 years (details in Text S1, Table S1). All stem vulnerability curves were fitted with a sigmoidal function (Vander Willigen & Pammenter 1998):

$$\text{PLC} = \frac{1}{1 + e^{\left(\frac{\text{slope}}{25}(\Psi_{\text{plant}} - \Psi_{50})\right)}} \quad (1)$$

where PLC is the percent loss of plant hydraulic conductance due to embolism,  $\Psi_{\text{plant}}$  is the xylem water potential,  $\Psi_{50}$  is the water potential causing a 50% loss of plant hydraulic conductivity and *slope* (%/MPa) is a shape parameter describing

the rate of embolism spread per unit water potential drop at the  $\psi_{50}$ . The  $\psi_{12}$  can be calculated from  $\psi_{50}$  and *Slope* ( $\psi_{12} = \psi_{50} + 50/\text{Slope}$ ). For all the species for which data for stem embolism resistance traits are available, we collected data for different traits indicating the level of plant water deficit ( $\Psi$ ) causing the highest degree of stomatal closure (hereafter referred to as  $\Psi_{\text{close}}$ ). We first used concomitant measurements of gas exchange and leaf water potential, from which the  $\Psi$  value at 90% stomatal closure was calculated as previously described (Klein 2014; Mencuccini *et al.* 2015; Bartlett *et al.* 2016). Stomatal opening increases with guard cell turgor pressure (Franks *et al.* 1998; Buckley 2005), and it has been shown that stomatal closure in woody species is largely explained by losses of leaf turgor (Brodribb *et al.* 2003; Rodriguez-Dominguez *et al.* 2016). We therefore also used leaf water potential at turgor loss ( $\Psi_{\text{tlp}}$ ) as a surrogate for  $\Psi_{\text{close}}$ , when  $\Psi_{\text{gs90}}$  was not available (see details on data acquisition in Text S2). Embolism resistance and  $\Psi_{\text{close}}$  data were collected from plants at all stages of development, but a comparison between small (mostly seedlings) and large plants (mostly adults) indicated that data for plants of different statures were comparable, at least in the context of a meta-analysis (Text S3, Figure S1).

We studied the statistical associations between the two different types of traits by fitting three different models (linear, sigmoidal and segmented models, Text S4). The segmented model provided the best fit to the data based on AIC (Table S2) and was retained for subsequent analyses. First, the fitted segmented regression between  $\Psi_{\text{close}}$  (or its component  $\Psi_{\text{tlp}}$  or  $\Psi_{\text{gs90}}$ ) and embolism resistance ( $\Psi_{50}$  or  $\Psi_{12}$ ) was used to identify (1) the break points in the  $x$  axis (i.e. the embolism resistance value at which there is a change in the covariation between  $\Psi_{\text{close}}$  and embolism resistance) and (2) the  $y$  axis intercept for this break point (i.e. the global limit for  $\Psi_{\text{close}}$ ). Second, we calculated the correlation coefficient and the linear regression between  $\Psi_{\text{close}}$  and embolism resistance for the data on either side of the break point. In addition to the results reported in the main manuscript, we provide separate analyses for gymnosperms and angiosperms and for each trait ( $\psi_{50}$ ,  $\psi_{12}$ ,  $\psi_{\text{gs90}}$  and  $\psi_{\text{tlp}}$ ) in Table S2, Table S3, Figure S3 and Figure S4. All the parameters used in this study are provided in a supplementary Excel file ‘*Database.xlsx*’.

### SUREAU MODEL: DESCRIPTION, SIMULATION AND VALIDATION

SurEau is a simplified discrete-time soil–plant hydraulic model used to simulate the time to hydraulic failure for the range of embolism resistance values reported in our database, under different hypotheses concerning the stomatal regulation of transpiration. SurEau assumes that plant death in severe drought conditions is due to desiccation, which is modelled through the process of cavitation. The system has been simplified to consider only two resistances (rhizosphere and plant), making it easy to apply, with only one stem vulnerability curve and no need for assumptions concerning hydraulic segmentation, a phenomenon dependent on mechanisms that remain a matter of debate (Bouche *et al.* 2015, 2016a,b; Cuneo *et al.* 2016; Scoffoni *et al.* 2017; Skelton *et al.* 2017).

### Description of the SurEau model

SurEau calculates soil and plant water status and assesses embolism by assuming that liquid water flow through the soil–plant system is exactly compensated by gaseous water losses at the surface of the foliage of the plant (i.e. steady-state conditions). Our general approach is inspired by many previous studies (Whitehead & Jarvis 1981; Tyree & Sperry 1988; Sperry *et al.* 1998; Tuzet *et al.* 2003) and has already been shown to apply to large time steps (>1 day) and small plants (Rambal 1993; Tuzet *et al.* 2003). The model also assumes that leaf and air temperatures are equal, to avoid the need to describe leaf energy balance. We can therefore write:

$$E = g_l \times \text{VPD} = k_{sl} \times (\Psi_{\text{soil}} - \Psi_{\text{plant}}) \quad (2)$$

where  $E$  is transpiration,  $g_l$  is leaf conductance for vapour water, VPD is the vapour pressure deficit between air and leaf,  $\Psi_{\text{soil}}$  is the soil water potential,  $\Psi_{\text{plant}}$  is plant water potential and  $K_{sl}$  is the plant leaf area-specific hydraulic conductance over the soil to leaf pathway.  $g_l$  includes the stomatal, cuticular and boundary layer conductances of the leaf. The control of  $E$  through stomata is treated through several assumptions described below.  $k_{sl}$  was calculated as the result of two conductances in series:

$$k_{sl} = \frac{1}{\frac{1}{k_{\text{soil}}} + \frac{1}{k_{\text{plant}}}} \quad (3)$$

where  $K_{\text{soil}}$  is the hydraulic conductance of the soil-to-root surface pathway and  $K_{\text{plant}}$  is the hydraulic conductance of the whole plant (i.e. from the roots to the leaves).  $K_{\text{plant}}$  was allowed to vary only to account for the loss of hydraulic conductivity caused by xylem embolism (Tyree & Ewers 1991):

$$k_{\text{plant}} = k_{\text{Pini}}(1 - \text{PLC}) \quad (4)$$

where  $K_{\text{Pini}}$  is the initial (i.e. pre-drought) plant hydraulic conductance and PLC is the percent loss of plant hydraulic conductance due to xylem embolism. PLC is calculated at each time step from the sigmoidal function for the vulnerability curve (VC) for embolism (see eqn 1).

We considered two different plant water reservoirs (Tyree & Yang 1990). The first, the apoplastic reservoir, consists of the inelastic xylem cells that release their water to the transpiration stream following embolism. This reservoir accounts for a large proportion of the water in stems [ $> 80\%$ , (Tyree & Yang 1990)] and is thought to be an important parameter for plant survival during drought episodes (Tyree & Yang 1990; Hölttä *et al.* 2009). The water freed by air filling feeds the water stream of the system, thereby tempering the decrease in water potential (Hölttä *et al.* 2009). As suggested by Hölttä *et al.* (2009), we considered any change in PLC to be followed by a proportional change in the volume of water released back to the system:

$$W_{xv} = V_X \times \text{PLC} \quad (5)$$

where  $W_{xv}$  is the amount of water released to the system and  $V_X$  is the total water-filled xylem volume of the plant ( $\text{m}^3$ ) and PLC is defined in eqn 1.  $V_X$  was calculated as:

$$V_X = E_{\text{md}} \times LA \times G \times \alpha_f \quad (6)$$

where  $E_{\text{md}}$  is the maximum diurnal transpiration (calculated from the maximal transpiration rate and assuming 10 h of transpiration per day),  $LA$  is leaf area,  $\alpha_f$  is the apoplastic fraction of the plant and  $G$  is the ratio of the total amount of water in the tree to maximum daily transpiration. The second reservoir considered was that formed by the elastic water release due to symplasm dehydration (i.e. the water released by the symplastic tissue  $1 - \alpha_f$ ). The dynamics of this reservoir depend on osmotic potential and the elasticity of the cell walls, which may either stretch or contract to allow water to flow in or out with changing  $\psi$ . This reservoir therefore constitutes an elastic form of storage, in which variation occurs at relatively high water potential (typically  $> -3$  MPa, Tyree & Yang 1990) and it can be described by pressure volume curves combined with the same formula as for cavitation (eqn 5). Symplastic water volume was thus calculated as in eqn 6 but with the symplastic fraction ( $1 - \alpha_f$ ) of the plant. The release of water from the symplastic reservoir ( $W_{sv}$ ) was computed as in eqn 5, with PLC replaced by the relative water content of the symplasm ( $R_s$ ).  $R_s$  was calculated from  $\Psi_{\text{leaf}}$  by inverting the classical pressure–volume curve equations (Appendix S3).

Variations of soil and rhizosphere conductance ( $K_{\text{soil}}$ ), and mean soil water potential in the root zone are calculated with van Genuchten–Mualem equations (Mualem 1976; van Genuchten 1980), from the unsaturated hydraulic conductivity of the soil ( $k_{\text{soil}}$ ), scaled to the rhizosphere according to the Gardner–Cowan formulation (Gardner 1964; Cowan 1965). Rhizosphere conductance can be expressed as:

$$K_{\text{soil}} = B \times k_{\text{soil}}(\Theta) \quad (7)$$

where  $k_{\text{soil}}$  is the unsaturated hydraulic conductivity of the soil at a given water content ( $\Theta$ ) or water potential (see below) and  $B$  is the root density conductance factor accounting for the length and geometry of the root system.  $B$  is based on the implicit assumption of a uniform root distribution in a soil layer, according to the Gardner–Cowan formulation (Gardner 1964; Cowan 1965).  $B$  is also called the ‘single root’ approach (Tardieu *et al.* 1992) as it is equivalent to assuming that plant water uptake occurs from a unique cylindrical root that has access to a surrounding cylinder of soil:

$$B = \frac{2\pi L_a}{\ln\left(\frac{b}{r}\right)} \text{ with } b = \frac{1}{\sqrt{\pi L_v}} \quad (8)$$

where  $L_a$  is the root length per unit area,  $r$  the mean root radius, and  $b$  is half the mean distance between neighbouring roots.  $b$  can be evaluated from  $L_v$ , the root length per unit soil volume.  $k_{\text{soil}}$  decreases with decreasing  $\Psi_{\text{soil}}$  because of the displacement of water from pores by air, as the capillary forces linking water to soil particles fail with increasing tension, thus creating dry non-conductive zones in the rhizosphere. Van Genuchten’s parametric formulation (van Genuchten 1980) for the water retention curve was used together with the equation of Mualem (1976) to calculate  $\Psi_{\text{soil}}$  and the unsaturated hydraulic conductivity of the soil as a function of soil relative extractable water content ( $\Theta$ ).  $\Psi_{\text{soil}}$  can be calculated as follows:

$$\Psi_{\text{soil}} = \frac{\left(\left(\frac{1}{\Theta}\right)^{\frac{1}{m}} - 1\right)^{\frac{1}{n}}}{\alpha}; m = 1 - \frac{1}{n} \quad (9)$$

where  $m$ ,  $n$  and  $\alpha$  are empirical parameters describing the typical sigmoidal shape of the function. Mualem (1976) provided a formula for changes in hydraulic conductivity with soil water content  $k_{\text{soil}}(\Theta)$ :

$$k_{\text{soil}} = k_{\text{sat}} \Theta^l \times \left[1 - \left(1 - \Theta^{\frac{1}{m}}\right)^m\right]^2 \quad (10)$$

where  $k_{\text{sat}}$  is the saturated hydraulic conductivity,  $l$  is a parameter describing the pore structure of the material (usually set to 0.5), and  $m$  is again set as in eqn 9. The relative extractable water content ( $\Theta$ ) is expressed as follows:

$$\Theta = \frac{\theta - \theta_r}{\theta_s - \theta_r} \quad (11)$$

where  $\theta$  is the relative water content (soil water content per unit soil volume),  $\theta_s$  is the relative soil water content at saturation (or field capacity) and  $\theta_r$  is the relative soil water content at wilting point.  $\theta_s$  and  $\theta_r$  are parameters measured in the laboratory or derived from soil surveys with pedotransfer functions. By contrast,  $\theta$  is variable, changing dynamically with changes in absolute soil water reserve in the rooting zone (WR). The parameters and the sensitivity analysis are provided in Appendix S4.

### Dynamic simulations

Under well-watered conditions, transpiration ( $E$ ) is forced at a constant value, assuming a constant high vapour pressure deficit. At each time step, the soil water reserve (WR) is calculated and then used to calculate all the other variables. WR is then calculated as the result of water balance:

$$WR_{t+1} = WR_t - E + W_{xv} + W_{sv} \quad (12)$$

where  $E$  is the cumulative transpiration over the time step,  $W_{xv}$  is water release due to cavitation and  $W_{sv}$  is water release due to symplasm dehydration (eqns 5 and 6 and the corresponding text). The time step was set to 0.1 days, but increasing this value to 0.5 or decreasing it had little influence on the general pattern of the results obtained.  $E$  was calculated as follows:

$$E = [E_{\text{max}} \times f(\psi_{\text{plant}})] \times LA \quad (13)$$

where  $E_{\text{max}}$  is the maximal transpiration rate,  $LA$  is plant leaf area and  $f(\psi_{\text{plant}})$  is the stomatal regulation function, which was set according to various hypotheses, as described below. The calibrations for  $E_{\text{max}}$ ,  $LA$ , and all the other parameters are provided in Appendix S4.

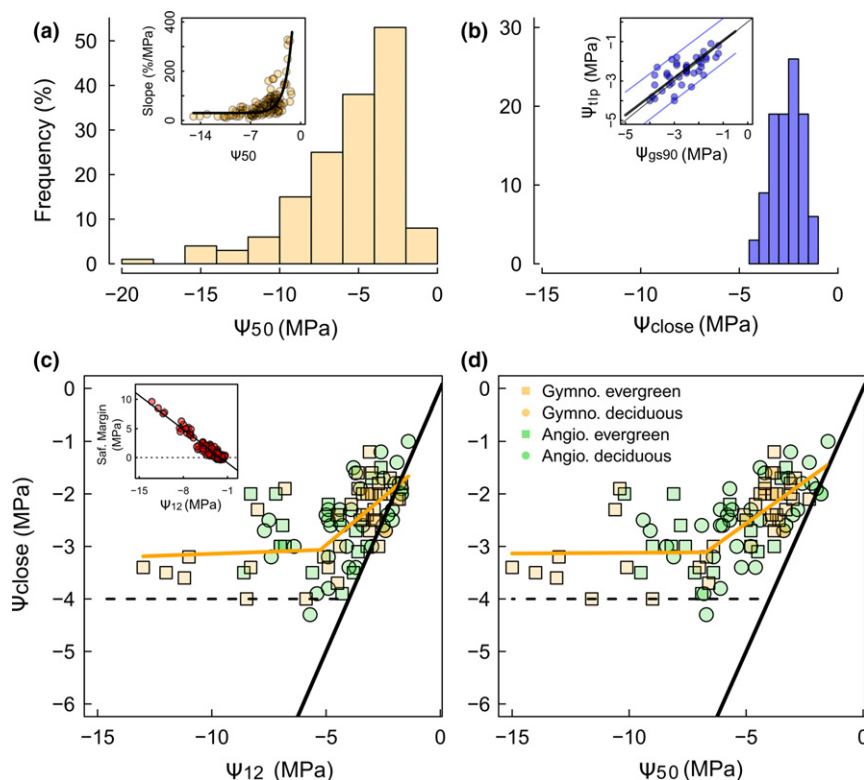
### Testing of hypotheses concerning the relationship between $\psi_{\text{close}}$ and $\psi_{50}$

We used the model described above to evaluate the role of  $\psi_{\text{close}}$  and  $\psi_{50}$  in determining survival time until hydraulic failure under drought conditions for the full range of embolism resistance reported in the database. Three different hypotheses

regarding the transpiration-regulating function of stomata were tested to obtain insight into the ways in which interplay between stomatal closure and embolism resistance shape survival under drought conditions (Fig. 2a). For the baseline simulation, we assumed that stomata never close ( $\psi_{\text{close}} = -\infty$ ), and thus do not regulate transpiration ( $E$ ) at all during drought, whatever the soil and plant water potential. This simplistic assumption was used to assess the effect of stomatal closure developed in hypotheses 2 and 3, described below. The second hypothesis tested was the widely accepted idea that stomata must close as soon as an embolism forms (corresponding to a tight match between  $\psi_{\text{close}}$  and  $\psi_{12}$ ). This hypothesis was modelled by assuming that the point at which leaf turgor is lost corresponds to the water potential causing incipient embolism (i.e.  $\psi_{\text{close}} = \psi_{12}$ ) over the entire embolism resistance spectrum.  $E$  regulation was modelled assuming progressive stomatal closure with the loss of leaf turgor, with a quadratic solution for pressure–volume equations (Vitali *et al.* 2016; Appendix S3). Once turgor is lost, the stomata are fully closed and  $E$  is reduced to a residual component corresponding to cuticular losses and stomatal leakiness due to imperfect closure (Brodribb *et al.* 2014). Finally, in our third hypothesis, the stomata regulate  $E$  in a timely manner following the onset of drought, such that  $\psi_{\text{close}}$  covaries with  $\psi_{12}$  until  $\psi_{\text{close}} = -3$  MPa, in accordance with the typical pattern reported for empirical data (see Results, Fig. 1c). Stomatal closure was simulated through turgor loss as in hypothesis 2. For each of these hypotheses, survival (taken as the number of days before 100% PLC is reached) was calculated for the full range of embolism resistance encountered in our database, (i.e. from  $\psi_{50} = -1.5$  to  $\psi_{50} = -19$  MPa, in 1 MPa steps). A detailed analysis of these simulations is provided in Appendix S2.

### Model validation: survival during drought, based on drought mortality experiments

For validation of the simulated relationship between survival and  $P_{50}$ , we built an empirical relationship between the survival measured in drought mortality experiments and embolism resistance. We collected mortality data for 15 species, covering a wide range of embolism resistance ( $\psi_{50}$  from  $-1.5$  to  $-11$ ) from four different drought mortality experiments published in recent years (Brodribb & Cochard 2009; Barigah *et al.* 2013; Urli *et al.* 2013; Li *et al.* 2015). One study concerned gymnosperm species only (Brodribb & Cochard 2009) and three other studies were performed on angiosperm species only (Barigah *et al.* 2013; Urli *et al.* 2013; Li *et al.* 2015). All these experiments were conducted under semicontrolled conditions, on seedlings or saplings in pots, and the drought treatment consisted of a cessation of watering until death. All studies recorded mortality estimated visually as the percentage of leaf or shoot death at various time points in the experimental drought period. We calculated the average time taken to reach 50% shoot death ( $T_{50}$ ) since the last watering in these studies, which we used as an indicator of survival during drought. Soil volume and climate were identical for all species in each experiment. However, the relative humidity of the air and soil volume differed between experiments (both these factors can strongly affect survival time during an episode of



**Figure 1** Range of variation of embolism resistance ( $\Psi_{50}$ ) and stomatal response to drought ( $\Psi_{close}$ ), and their covariation. (a) Distribution of embolism resistance ( $\Psi_{50}$ ) among plants. The inset shows the relationship between the *slope* of the vulnerability curve (%/MPa, Text S1) and  $\Psi_{50}$ , making it possible to calculate  $\Psi_{12}$  (MPa). The best fit was obtained with  $slope = 16 + e^{(\Psi_{50})} \times 1092$ , the fitted parameters were significantly different from 0 ( $P < 0.01$ ). (b) Distribution of the water potential causing stomatal closure ( $\Psi_{close}$ ). The relationship between the two different traits for  $\Psi_{close}$  (the water potential causing 90% stomatal closure,  $\Psi_{gs90}$ ) and the water potential causing leaf turgor loss ( $\Psi_{tip}$ ) is shown in the inset with the fitted line ( $\Psi_{tip} = 0.97 \times \Psi_{gs90}$ ,  $P < 0.01$ ,  $R^2 = 0.4$ ) and the 95% confidence interval. (c) The relationship between  $\Psi_{close}$  and  $\Psi_{12}$ . (d) Relationship between  $\Psi_{close}$  and  $\Psi_{50}$ . The 1:1 line (continuous line) and the quantile 99% (dashed line) are shown. The orange line is the best fit with a segmented regression, showing a significant breakpoint for  $\Psi_{50}$  of  $-5.9$  MPa or  $\Psi_{12}$  of  $-4.2$  MPa corresponding to an average  $\Psi_{close}$  of about  $-3$  MPa ( $\pm 1.5$  MPa, 95% CI). The inset in (c) shows the linear relationship between the safety margins between stomatal closure and embolism formation ( $\Psi_{close} - \Psi_{12}$ ) and  $\Psi_{12}$  ( $P < 0.01$ ). In (c) and (d), the points correspond to individual species, with pictograms highlighting the different functional and taxonomic groups, as indicated in the legend to (d).

water deficit), precluding direct comparisons of survival between the four studies considered. We therefore used (1) a generalised mixed-effects model showing a significant effect of  $\Psi_{50}$  on  $T_{50}$  ( $P = 0.0003$ ) and a significant interaction between  $\Psi_{50}$  and experiment (study) ( $P = 0.0128$ ) (Appendix S1), and (2) a standardised  $T_{50}$  for each experiment taking into account the differences in soil volume (Appendix S1).

## RESULTS AND DISCUSSION

Embolism resistance (taken as  $\Psi_{50}$ ) ranged between  $-1.3$  and  $-19$  MPa (Fig. 1a). The large variations of  $\Psi_{50}$  were partly related to changes in *slope*, which was non-linearly related to  $\Psi_{50}$  (Fig. 1a, inset).  $\Psi_{50}$  and *slope* together determined the water potential causing the onset of embolism ( $\Psi_{12}$ ). This more conservative indicator of embolism resistance ranged between  $-0.7$  and  $-14$  MPa. The two indicators of water potential causing stomatal closure ( $\Psi_{close}$ ) were significantly related to each other, with a slope close to one ( $P < 0.01$ ; Fig. 1b, inset), as previously reported (Mencuccini *et al.* 2015; Bartlett *et al.* 2016),  $\Psi_{close}$  was thus taken as the average value when the two traits were available.  $\Psi_{close}$  varied from  $-1$  to

$-4.3$  MPa, spanning a range of variation only one-third that for embolism resistance (Fig. 1a and b), consistent with the findings of recent meta-analysis (Mencuccini *et al.* 2015; Bartlett *et al.* 2016).

Our meta-analysis showed that most species have  $\Psi_{close}$  values that are higher than their  $\Psi_{50}$  or  $\Psi_{12}$  values (Fig. 1c and d). The difference between  $\Psi_{50}$  and  $\Psi_{close}$ , defined as the safety margin between stomatal closure and embolism formation ( $SM_{P50}$ ) was greater than zero for 99% of the species studied, and  $SM_{P12}$  was greater than zero for 67% of the species studied (Fig. S2). This finding is not consistent with the expected coordination between stomatal closure and embolism resistance based on small safety margins to ensure the maintenance of  $CO_2$  assimilation for as long as possible during drought (Tyree & Sperry 1988). The 1:1 line (in Fig. 1c and d) therefore appears to define a first boundary to plant hydraulics. We also found that safety margins increased continuously with increasing embolism resistance (Fig. 1c inset). This relationship reflects the low slope value of 0.4 for species with low embolism resistance and the segmented relationship between  $\Psi_{close}$  and embolism resistance for high embolism resistance (Fig. 1c and 1d, summary statistics and

comparisons with other models are provided in Table S2). Break-points for the segmented relationships were found for  $\Psi_{12} = -4.5$  and  $\Psi_{50} = -6$  MPa, corresponding to a  $\Psi_{\text{close}} = -3$  MPa on average (Fig. 1c and d and Table S3).  $\Psi_{\text{close}}$  reached a plateau at  $-4$  MPa (1st percentile of the distribution), even for species highly resistant to embolism. A second boundary is thus defined by the limit of  $\Psi_{\text{close}}$ . Overall, these patterns were also observed within the different taxonomic (gymnosperm and angiosperm) and functional (evergreen and deciduous) groups tested and they were not affected by consideration of the two indicators for  $\Psi_{\text{close}}$  separately (Fig. 1c and d, Figure S3, Figure S4, Table S3).

The vascular system of terrestrial plants has evolved towards very high levels of embolism resistance (Delzon *et al.* 2010; Pittermann *et al.* 2012; Larter *et al.* 2017), reaching  $\Psi_{50}$  values down to  $-19$  MPa, which is close to the practical limit of water metastability, suggesting that liquid water transport under the cohesion-tension theory has reached its operational boundary in these aridity-resistant species (Larter *et al.* 2015). It could be hypothesised that stomatal closure has evolved along similar lines, to maintain gas exchange (e.g. for carbon assimilation and transpiration) for longer periods during drought, even at low xylem water potential. However, our findings conflict with this view, suggesting that stomatal closure is subject to additional constraints. A physiological limit to stomata opening at low water potential may arise due to the deleterious effects of the solute accumulation in leaves (i.e. osmotic adjustment) required to maintain turgor pressure and stomatal opening. Excessive solute accumulation may lead to precipitation, severely impairing protein activity. However, van't Hoff's law predicts solute precipitation at osmotic potentials far below ( $< -10$  MPa for KCl or sugars) the values of  $\Psi_{\text{close}}$  reported in our database. This uncoupling of stomatal closure and embolism resistance may alternatively result from selection pressures that have favoured survival under extreme water scarcity over growth under mild drought conditions. This greater safety margin between stomatal closure and embolism formation would have allowed plants to adapt to extreme drought conditions and to colonise xeric environments.

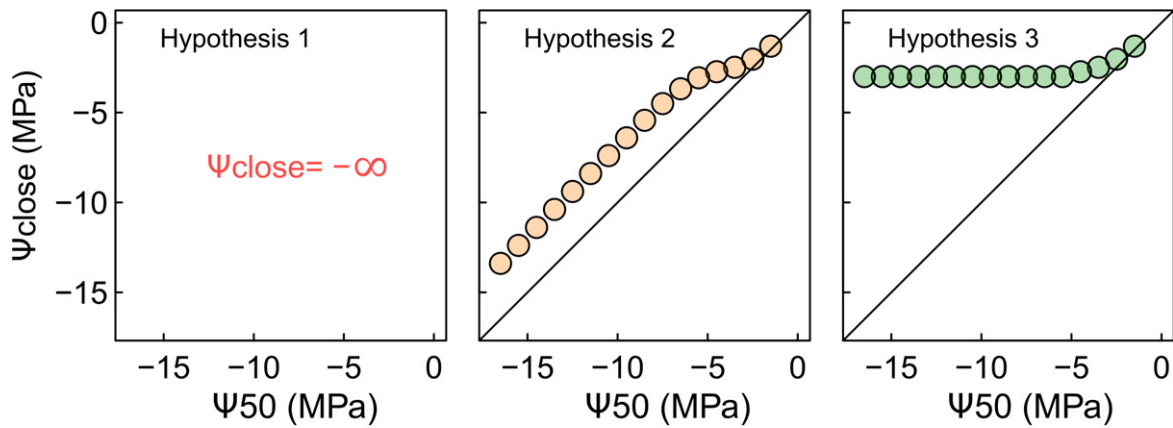
We investigated this pattern further, using the SurEau model to calculate survival times under drought conditions (i.e. the time to reach 100% loss of hydraulic conductance) for a range of hypothetical species covering the full spectrum encountered in our database. Three hypotheses for the stomatal regulation of  $E$  were used to evaluate how the interplay between stomatal closure and embolism resistance shapes survival under drought conditions (Fig. 2a, see Methods and Appendix S2). Under the hypothesis of no stomatal closure during drought (i.e. Hypothesis 1, Fig. 2a), plants would die very rapidly from hydraulic failure, with only a slight increase in survival when  $\Psi_{50}$  decreased from  $-1$  to  $-6$  MPa (Fig. 2c). For  $\Psi_{50}$  values below  $-6$  MPa, increasing embolism resistance was not associated with a further increase in survival time. Despite their simplicity, these control simulations indicate that increasing embolism resistance *per se* has only a marginal impact on survival under drought conditions, particularly for highly resistant species.

Under the assumption that stomata should close at the onset of embolism (i.e. Hypothesis 2, where  $\Psi_{\text{close}} = \Psi_{12}$ , Fig. 2b), a much higher mean survival time was obtained (Fig. 2b). Survival also increased markedly with embolism resistance until a  $\Psi_{50}$  of  $-6$  MPa. However, beyond this value, survival decreased substantially (Fig. 2b), contrary to the trend observed in analyses of experimentally induced mortality (Fig. 2c). This increase in survival at high  $\Psi_{50}$  ( $\Psi_{50} > -6$  MPa) values is due to the maintenance of  $\Psi_{12}$  (and thus of stomatal closure) at an almost constant value of about  $-3$  MPa, due to the decrease in the *slope* of the VC with decreasing  $\Psi_{50}$  (Appendix SA2–4, Fig. A2–6). Under this assumption, if the *slope* of the VC was maintained at a constant mean value for all the values of  $\Psi_{50}$  tested, the model would simulate a continuous decrease in survival for all levels of  $\Psi_{50}$  tested (Appendix S2–2, Fig. A2–2). The survival peak simulated at a  $\Psi_{50}$  of  $-6$  MPa (Fig. 2b) under this hypothesis implied a  $\Psi_{\text{close}}$  value of about  $-3$  MPa, corresponding to the mean limit for  $\Psi_{\text{close}}$  in our database. Thus, assuming that  $\Psi_{\text{close}}$  does not covary with embolism resistance beyond  $\Psi_{\text{close}} = -3$  MPa (hypothesis 3, Fig. 2a), a positive relationship between survival and embolism resistance was predicted over the entire range of  $\Psi_{50}$  (Fig. 2b), consistent with the empirical trend observed in drought mortality experiments (Fig. 2c). These simulations support the view that embolism resistance cannot increase survival unless the difference between embolism resistance and  $\Psi_{\text{close}}$  also increases.

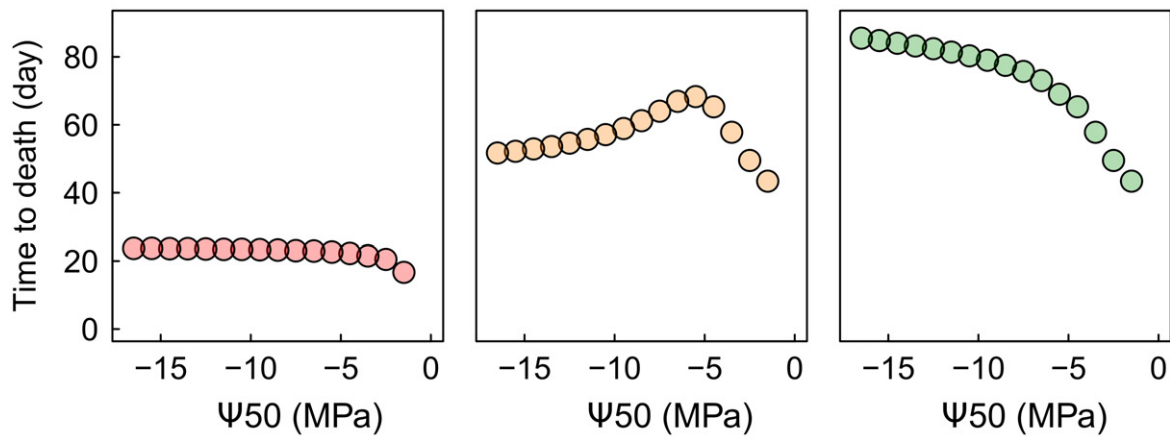
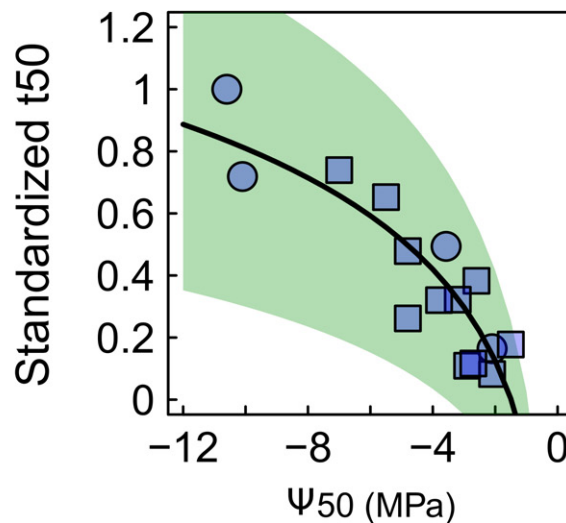
An analysis of the modelled dynamics of soil and plant dehydration for two species with contrasting levels of embolism resistance identified the physical mechanisms making early stomata closure necessary for the avoidance of drought-induced mortality, even for embolism-resistant species (Fig. 3). The relationship between soil water potential ( $\Psi_{\text{soil}}$ ) (and, hence, plant water potential) and soil water content ( $\theta$ ) becomes nonlinear at relatively high values of  $\Psi_{\text{soil}}$  (Fig. 3a and b). Thus, the longer transpiration is maintained, the sharper the rates of decrease in soil and plant water potential, leading to rapid death through hydraulic failure. The nonlinearity of the  $\Psi_{\text{soil}}$  ( $\theta$ ) relationship results from long-established physical laws (Campbell 1974; van Genuchten 1980) describing the changes in  $\Psi_{\text{soil}}$  and soil conductivity with soil water content. These laws are globally conserved among soil types (Appendix A4, Figure A4–1), providing support for the overall scope of our findings.

The vascular system of terrestrial plants has evolved towards very high levels of embolism resistance ( $\Psi_{50}$  values down to  $-19$  MPa), enabling plants to colonise dry environments (Pittermann *et al.* 2012; Larter *et al.* 2017). Stomatal closure might have been expected to have evolved along similar lines, to maintain carbon assimilation levels for longer periods, even at low xylem water potential. Moreover, several recent studies have reported close covariation between stomatal closure in response to drought and embolism resistance, but only for species with relatively low levels of drought resistance (Cruziat *et al.* 2002; Klein 2014; Mencuccini *et al.* 2015; Bartlett *et al.* 2016). Our results indicate that the range of variation of  $\Psi_{\text{close}}$  is much smaller when considered in the light of the full range of embolism resistance. This uncoupling of stomatal closure and vascular system failure may result

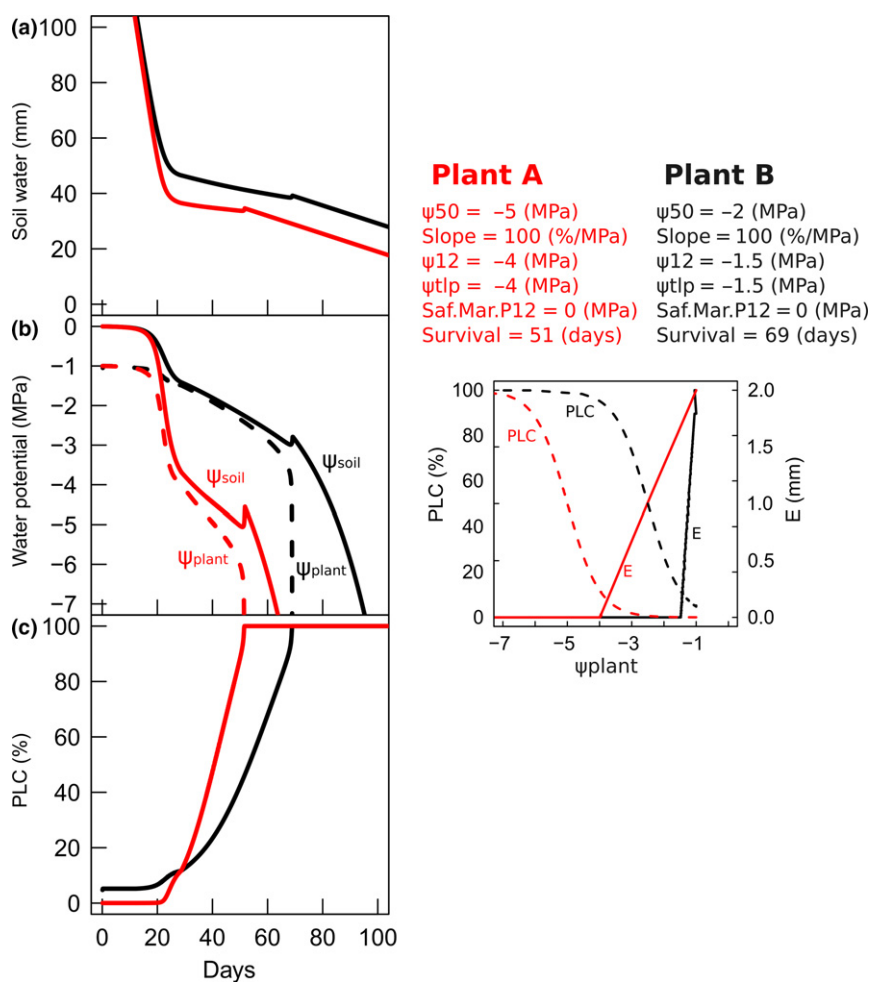
## (a) Hypothetical stomatal behavior



## (b) Model results

(c) Data of time to 50% canopy death ( $t_{50}$ )

**Figure 2** Model simulations of survival time for the full range of embolism resistance, under three different hypotheses concerning stomatal behaviour. (a) Representation of the parameter combinations for  $\Psi_{\text{close}}$  and  $\Psi_{50}$  used to represent the three hypotheses tested in the model: (Hypothesis 1) Stomata never close (i.e. plants maintain maximal rates of transpiration at all soil and plant water potentials, whatever their  $\Psi_{50}$ ). Hypothesis 1 is used as a control, to assess the effect of stomatal closure under the other hypotheses. (Hypothesis 2) Stomata gradually close with turgor loss, such that water potential at full closure ( $\Psi_{\text{close}}$ ) equals  $\Psi_{12}$  (i.e. tight coordination between  $\Psi_{\text{close}}$  and  $\Psi_{12}$ ). (Hypothesis 3) Stomatal closure and embolism resistance are equal only to  $-3$  MPa, as indicated by our empirical results (Fig. 1). (b) Simulated relationship between survival time (time to reach 100% of PLC) and  $\Psi_{50}$  for each hypothesis tested. With the exception of the changes to E regulation made to satisfy the hypothesis tested, all other parameters were kept constant (Appendix S4). (c) Normalised time to 50% shoot death ( $T_{50}$ ) as a function of  $\Psi_{50}$  for 15 species. The data were collected from four different studies and normalised to account for differences in soil and climate conditions across experiments (see Methods). The logarithmic relationship fitted to absolute values ( $0.42 \times \log(|\Psi_{50}|) - 0.16$ , slope  $P < 0.001$ ) is shown (line) with its 95% CI (green area).



**Figure 3** Simulated temporal dynamics of soil and plant dehydration assuming that stomata gradually close to reach full closure at  $\Psi = \Psi_{12}$  (hypothesis 2) for two species with different stomata and hydraulic traits, as described in the right panel. (a) Soil water content, (b) soil and plant water potential and (c) the percent loss of conductivity caused by embolism. Simulations were performed for two hypothetical plants (plant a and plant b), the traits of which are shown in MPa on the plot. The time to death from hydraulic failure (i.e. 100% embolism) is also indicated above the right panel. Simulations show that higher levels of embolism resistance and, thus, higher water potential at full stomatal closure accelerate death, due to a faster decrease in water potential. More detailed simulation results are provided in Appendix S2.

from selection pressures that have favoured survival under conditions of extreme water scarcity over growth under mild drought conditions.

These findings provide a view complementary to the widely accepted framework for drought response strategies based on the water-to-carbon trade-off [e.g. (McDowell *et al.* 2008; Skelton *et al.* 2015; Yoshimura *et al.* 2016)]. According to this framework, plant drought response strategies lie between two extreme categories: isohydric and anisohydric (McDowell *et al.* 2008; Klein 2014; Martínez-Vilalta *et al.* 2014). Isohydric plants close their stomata rapidly in response to drought, thereby maintaining a high water potential to limit embolism, but at the risk of death due to carbon starvation. Conversely, anisohydric plants keep their stomata open at low water potential, maintaining carbon assimilation levels, but at the cost of damage to the water transport system due to embolism. This framework has been the focus of many scientific studies on drought-induced mortality in recent decades and underpins the current understanding and modelling of drought-induced plant mortality (McDowell *et al.* 2008; Skelton *et al.* 2015;

Yoshimura *et al.* 2016). The finding that the most drought-resistant plants close their stomata at a potential much higher than that at which embolism can occur indicates that drought resistance may not involve the maintenance of gaseous exchanges during drought conditions. Indeed, it demonstrates that, on the contrary, plants have to limit decreases in water potential, as confirmed by the modelling analysis (Fig. 2b).

The relatively low variation of  $\Psi_{close}$  relative to  $\Psi_{50}$  may appear to conflict with the large variations in minimum water potential reported by various studies (Choat *et al.* 2012; Martínez-Vilalta *et al.* 2014; Anderegg *et al.* 2016; Martínez-Vilalta & Garcia-Forner 2016). However, it may highlight the importance of accounting for the multiple traits driving the demand for water when stomata are closed in representations of water potential decline and, thus, plant dehydration. For instance, the minimum leaf conductance (i.e. when stomata are closed) and leaf area are important traits driving plant water potential decline. The hydraulic model presented here is consistent with this view. Accordingly, model simulations indicated that there were two main stages defining the temporal



sequence leading to plant desiccation in situations of water scarcity (Fig. 3). The first of these stages is defined by the time between the start of water shortage and stomatal closure. Its duration depends principally on the rate of water uptake, given the relative constancy of  $\Psi_{\text{close}}$  in plants and the competition between plants for water in community ecosystems. The second stage is defined by the time between stomatal closure and plant death (100% embolism). The duration of this stage depends on a set of drought resistance traits allowing plant tissues to retain water under very high tension, to decrease water loss when the stomata are closed and to limit the decrease in water potential during embolism through deeper rooting or the release of water from internal stores (Blackman *et al.* 2016). It remains to be seen how these other traits covary with embolism resistance, are coordinated and have coevolved to shape the spectrum of drought adaptation strategies in plants. However, the conclusions drawn here at the plant scale may require adjustment at a later stage, when improvements in experimental methodology allow reliable measurements of leaf and fine root xylem vulnerability to be incorporated into the model.

Overall, the model analysis presented here demonstrates that multiple measurable drought resistance traits can be integrated into a consistent and thermodynamically reliable formal framework to define drought-induced mortality (Pivovarov *et al.* 2016). This modelling approach must be validated carefully against the temporal dynamics of water potential, hydraulic conductance, data for embolism proper, and experimental and field mortality for different species. The model will need to evolve with advances in our understanding of plant hydraulics, to explore mechanisms that are expected to be important for plant survival, such as hydraulic segmentation, the role of plant capacitance or the impact of cuticular transpiration on energy balance, critical leaf temperatures and plant desiccation. However, in its present form, it constitutes an important step in assessments of the consequences of drought in land plants and the effects of climate change on terrestrial ecosystem functions. It may also prove to be a powerful tool for taking multiple traits into account in breeding strategies.

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#### AUTHORS' CONTRIBUTIONS

NM, SD and HC conceived the idea for this work. NM assembled the data set and analysed the data with inputs from

SD. HC developed a preliminary version of the SurEau model. NM implemented the model under R and performed the computational analysis. NM wrote the manuscript with revisions from SD and HC.

#### DATA ACCESSIBILITY

The Database is fully available in zenodo repository: DOI: 10.5281/zenodo.854700 (<https://zenodo.org/record/854700#.Wabc-ulpuyU>)

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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