Drought will not leave your glass empty: Low risk of hydraulic failure revealed by long-term drought observations in world’s top wine regions

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Grapevines are crops of global economic importance that will face increasing drought stress because many varieties are described as highly sensitive to hydraulic failure as frequency and intensity of summer drought increase. We developed and used novel approaches to define water stress thresholds for preventing hydraulic failure, which were compared to the drought stress experienced over a decade in two of the world’s top wine regions, Napa and Bordeaux. We identified the physiological thresholds for drought-induced mortality in stems and leaves and found small intervarietal differences. Long-term observations in Napa and Bordeaux revealed that grapevines never reach their lethal water-potential thresholds under seasonal droughts, owing to a vulnerability segmentation promoting petiole embolism and leaf mortality. Our findings will aid farmers in reducing water use without risking grapevine hydraulic integrity.

INTRODUCTION

Water availability is one of the most important factors in determining plant survival and productivity in both ecological and agricultural contexts (1). The consumption of freshwater resources for agriculture is enormous, especially in dry environments; for example, 80% of freshwater resources are used for agriculture in California. Thus, reducing crop water use is essential to increase agricultural sustainability. The need to reduce water use, paired with the increased likelihood of large-scale water deficits and extreme drought events (2), is driving the search for more drought-resistant crops. A cornerstone to this pursuit is integrated understanding of the physiological mechanisms driving the resilience of the plant hydraulic system, including water potential (Ψ) and stomatal regulation, vulnerability to embolism along the water column, and ability to recover from drought (3).

One thing is inescapable; as plant water status [that is, leaf water potential (Ψleaf)] declines, plants regulate stomata to control transpiration-water loss. Stomatal closure is one of the first responses to drought stress (4), protecting against loss of hydraulic conductivity by maintaining xylem pressure above the onset of embolism (5, 6). Scientists have used a framework to describe variation in stomatal control where a species may be defined as more isohydric or more anisohydric (7–10). Most commonly, iso/anisohydric behavior refers strictly to differences in the regulation of stomatal conductance in response to water deficit, assuming that stomatal conductance is assessed under identical environmental conditions across decreasing Ψ [for example, see study of Martínez-Vilalta et al. (9)]. But equally, iso/anisohydric behavior can also refer to the regulation of stomatal conductance in response to increasing vapor pressure deficit (D) (11–13). A more isohydric behavior is one where the plant maintains Ψ within a given range under water deficit and high evaporative demand through limiting transpiration. A more anisohydric behavior is one where the plant, to a certain extent, maintains transpiration under water deficit and high evaporative demand, tolerating increasingly negative Ψ. A more isohydric behavior avoids the immediate risk of xylem embolism formation by maintaining Ψ but consequently limits photosynthesis. In the ecological context, interspecific differences in iso/anisohydric behavior between forest trees correspond to differences in their performance and mortality under drought (14). However, this framework is currently debated even at the interspecific level (8–10), and to be of use for the breeding of agricultural crops, differences in iso/anisohydric behavior need to occur at the intraspecific (or intragenus) level.

Grapevines are crops for which different varieties supposedly exhibit contrasting behaviors within the iso/anisohydric paradigm [reviewed by Chaves et al. (15)]. The most classic examples are Grenache (isohydric) and Syrah [anisohydric (16)]. These contrasting behaviors are often invoked in a discussion of drought resistance, but it is not entirely clear which would be most beneficial in the context of a woody perennial crop where the iso/anisohydric paradigm constitutes a trade-off between safety with respect to hydraulic failure and productivity. Furthermore, the intraspecific iso/anisohydric behaviors among grapevine varieties can be difficult to separate (17), and individual grapevine varieties can exhibit contradictory behavior depending on the study (15).

Stomatal closure and reductions in transpiration should occur before the onset of embolism (3). Recent work in Vitis vinifera suggests that this is indeed the case, where stem embolism occurs at lower (more negative) Ψ inducing 50% loss of hydraulic conductivity (Ψ50) < −1.7 MPa than those typically observed for stomatal closure [Ψleaf < −1.0 MPa (18)]. The distance between the minimum stem water potential (Ψstem) experienced in the field (Ψmin) and the Ψ50 represents the hydraulic safety margin (19, 20).
the more susceptible a plant will be to hydraulic failure. For example, the width of the hydraulic safety margin has been related to differences in drought-induced mortality in trees (21). In perennial crops, the hydraulic safety margin could be important in longevity and long-term productivity under drought. To date, the hydraulic safety margin has not been directly assessed in grapevine, a trait that could potentially differ substantially between varieties exhibiting different strategies for regulation of plant water status.

The current study explores physiological differences in grapevine at the interspecies level by comparing the responses to extreme water deficit (that is, until death of the aboveground biomass) across *V. vinifera* and rootstock varieties. We developed an integrative approach to characterize the dynamic hydraulic strategies of these varieties during increasing drought stress that included the regulation of $\Psi_{\text{leaf}}$ (greenhouse versus field plants) and stomatal conductance (at the leaf and whole-plant scale in a greenhouse) in response to a decrease in soil water availability, to plant water status, and to an increase in evaporative demand while accounting for other confounding environmental parameters such as light level. The stem vulnerability to embolism was characterized along the growing season using different techniques [in situ flow centrifuge— and bench-based vulnerability curves, confirmed using high-resolution computed tomography (HRCT)]. For the first time, the safety margin of a range of grapevine varieties was quantified and compared to the in situ water stress experienced in the vineyard across more than a decade in two of the world’s most important wine regions.

**RESULTS AND DISCUSSION**

**Unraveling (an-)isohydric behavior by integrating responses to soil water deficit and vapor pressure deficit**

Assigning a determined behavior in $\Psi$ regulation is problematic in grapevine (15) and other species (22). *V. vinifera* cv. Grenache and Syrah have repeatedly been described as isohydric and anisohydric, respectively (16, 23). Thus, Grenache would be expected to maintain midday water potential ($\Psi_{\text{mdl}}$) at a higher level than Syrah under similar soil water status [estimated via the relative water content (RWC)] and/or evaporative demand [estimated via the vapor pressure deficit (D)]. Using a large data set of $\Psi$ monitoring across a wide range of drought conditions and combining field observations and greenhouse experiments, we explored the relationship between midday leaf ($\Psi_{\text{mdl}}$) and predawn water potential ($\Psi_{\text{pd}}$). These two variables did not exhibit significant differences in the relationship between $\Psi_{\text{mdl}}$ and $\Psi_{\text{pd}}$ (where the slope $\sigma$ represents the relative sensitivity of the transpiration rate and plant hydraulic conductance to declining water availability) (9), under field conditions ($P = 0.074$; Fig. 1) nor during a greenhouse drydown experiment ($P = 0.225$). Additional comparisons also did not reveal significant differences in the relationship between midday stem water potential ($\Psi_{\text{msd}}$) and $\Psi_{\text{pd}}$ among three varieties in the Languedoc and two varieties in Saint-Emilion ($P > 0.270$ and 0.068, respectively; fig. S1). Finally, the water use envelope (24) or “hydroscape” (19) that corresponded to the limit of $\sigma$ did not exhibit significant differences across varieties (1.32 and 1.38 MPa$^2$ for Grenache and Syrah, respectively; Fig. 1).

If (an-)isohydric behavior was genetically determined [for example, see the study of Coupel-Ledru et al. (25)], we would expect each variety to exhibit a consistent behavior regardless of the growing conditions. Under field conditions, both varieties exhibited extreme anisohydric behavior ($\sigma = 1.172 \pm 0.034$ MPa MPa$^{-1}$, mean ± SE; Fig. 1), whereas partially isohydric behavior was observed in potted plants ($\sigma = 0.861 \pm 0.025$ MPa MPa$^{-1}$, mean ± SE; $P < 0.001$). Synthesizing published studies on these varieties suggests a similar variability between field (16) and potted plants (26).

The ratio between belowground and aboveground biomass was substantially higher in the field than in the greenhouse and likely resulted in differences in accessing soil water. Consequently, the apparent behavior in response to drought may be related to the range of $\Psi$ explored by the plant. Compared to field conditions, greenhouse plants experienced much more negative $\Psi_{\text{pd}}$ ($-1.4$ and $-2.9$ MPa in the field and greenhouse, respectively). Therefore, we explored changes in $\sigma$ across an expanding range of $\Psi_{\text{pd}}$/$\sigma$. The slope $\sigma$ varied widely (top insets in Fig. 1). Under moderate stress ($\Psi_{\text{pd}} > -1.5$ MPa), both varieties behaved as extremely anisohydric ($\sigma_1 > 1$) and then anisohydric ($\sigma_1 = 1$) for $\Psi_{\text{pd}} = -1.5$ MPa, whereas they became partially isohydric ($\sigma_1 < 1$) under more severe levels of stress ($\Psi_{\text{pd}} < -1.5$ MPa). At moderate stress, Syrah exhibits a more pronounced anisohydric behavior according to $\sigma_1$, which converges with Grenache as stress levels increase. The current definition regarding the behavior of these varieties may be improved by representing the behavior as dynamic across the (an-)isohydric spectrum, representing an anisohydric to isohydric transition as stress increases.

Neither the dynamics of $\sigma_1$ nor the hydroscape framework (10) necessitates a linear curve fit. We also explored the framework with nonlinear fits, which improved the statistical significance of the fit [see the estimated Akaike information criterion (AIC); fig. S2], better represented the minimum $\Psi$, and suggested a slightly larger hydroscape for Syrah (1.42 MPa$^2$) compared to Grenache (1.17 MPa$^2$). This approach also revealed differences between Syrah and Grenache at high $\Psi$ ($\Psi_{\text{pd}} > -0.8$ MPa).

Traditionally, the stomatal response to drought stress has been used to assign the (an-)isohydric behavior (7, 14, 16). For instance, anisohydric plants could sustain longer periods of transpiration and photosynthesis under water scarcity, allowing them to be more drought-tolerant than isohydric species (27). If grapevine varieties would regulate $\Psi$ differently,
then it would be observed at the stomatal level as well. However, no significant differences across four contrasting varieties were observed in the decline of stomatal conductance in response to $\Psi_{\text{sat}}$ at the leaf ($g_s$) or whole-plant ($G_s$) scale ($P = 0.558$ and $0.164$ for $g_s$ and $G_s$, respectively; Fig. 2A and B). Equally, no difference was observed in response to $\Psi_{pd}$ ($P = 0.836$; fig. S3). Drawing firm conclusions about differences in leaf-level stomatal conductance (Fig. 2A) is difficult because of the intrinsic variability in the measurement, but this parameter was assessed to complement our whole-plant data (Fig. 2B), which is extremely robust across the full range of $\Psi$ (approximately 5000 data points per variety). These findings are consistent with the lack of differences in leaf intrinsic water-use efficiency reported between isohydic and anisohydric genotypes (17).

The transition from anisohydry to isohydry observed here would imply a stronger control of $g_s$ or $G_s$, especially to $D$, as drought stress increases. Using a lysimeter platform, we assessed the real-time whole-plant $G_s$ over a 2-month period, exploring the dynamic change in stomatal sensitivity to $D$ during increasing drought stress (fig. S4). To visualize this relationship between $G_s$ and $D$ as a function of plant water status, we used the framework established by Oren et al. (28) in which sensitivity to vapor pressure deficit [that is, $dG_s/\ln(D)$] is plotted against $G_s$ at 1-kPa vapor pressure deficit ($G_{\text{ref}}$) (Fig. 2C). In all varieties, $G_s$ did not decrease with increasing $D$ until $\Psi_{\text{sat}}$ was lower than $-0.5$ MPa (Fig. 2C, inset, and fig. S4). Dynamic changes in sensitivity revealed that the $\Psi$ threshold at which $G_s$ became sensitivity to $D$ was similar across varieties: $\Psi_{pd} = -0.72 \pm 0.03$ MPa ($-0.67, -0.69, -0.71$, and $-0.80$ MPa in Grenache, Syrah, V. riparia, and 110 Richter, respectively). Furthermore, when drought stress increased, the ratio between $G_{\text{ref}}$ and $s$ became constant, reaching the generic value of 0.6 that assumes tight stomatal regulation of $\Psi_{\text{sat}}$ across isohydric species (28).

These dynamics in stomatal regulation with respect to $D$ under decreasing water availability may explain the continuum between anisohydric and isohydric behaviors (8). Well-watered plants exhibited wide variations in $s$ because $\Psi_{\text{sat}}$ is mainly driven by daily variations in transpiration, and thus, by environmental conditions [that is, light, temperature, and $D$ (29)]. Although plants can exhibit differences under very moderate stress, these differences disappear as water stress increases ($\Psi_{pd} < -0.8$ MPa). These dynamic relationships with respect to soil water and $D$ likely explain the resulting confusion, with respect to either $\Psi$ or stomatal regulation, in assigning a single behavior. Understanding a variety’s behavior under drought requires examining stomatal responses with respect to both soil water and $D$ simultaneously across the full range of potential stress levels. Here, we present one possible framework to visualize and quantify soil water deficit/D dynamics in an integrated fashion.

In light of the data presented here, the traditional Grenache/Syrah iso/anisohydric contrast is more nuanced. The relationship between $G_{\text{ref}}$ and $s$ (Fig. 2C) suggests a more anisohydric behavior for Syrah, but these differences are slight and significant only when particular frameworks are used (that is, Figs. 1 and 2A and fig. S2). In the field, Syrah experiences slightly more negative $\Psi$ than Grenache (Fig. 1 and fig. S2), but at these levels of stress, $G_s$ is nearly zero, suggesting that these differences have to be due to factors other than stomatal regulation per se (for example, differences in cuticular transpiration and/or root hydraulic conductance). Approaches to breeding drought resistance in Vitis based solely on phenotyping differences in stomatal regulation appear insufficient and may benefit from using a more detailed, holistic, and integrative approach like the one used in this study.

**Stem vulnerability is dynamic, closely following drought intensity along the season**

Despite the fact that *V. vinifera* is adapted to environments experiencing seasonal drought, studies differ in concluding whether it is sensitive (30) or resistant to embolism (31). Because of the similarity in regulating $\Psi$ and stomatal conductance described above, a variety’s hydraulic safety margin would ultimately depend on its xylem vulnerability. Therefore, we may expect contrasting varieties to exhibit significant differences in this drought-related trait. Long stems from different varieties were centrifuged to mimic drought stress and induce loss of hydraulic conductivity using the in situ flow centrifuge technique equipped with a 1-m-large rotor. Contrary to all expectations, no significant differences were observed across *V. vinifera* varieties ($P = 0.67$ and 0.09 in July and September, respectively; Fig. 3, A and B).
These results were confirmed using the bench dehydration method combined with the gravimetric method (Fig. 3C). Different *V. vinifera* varieties consequently exhibit a similar hydraulic vulnerability.

Drought exposure (that is, minimum $\Psi_{stem}$) is dynamic over the course of the growing season, although no compensatory seasonal changes in stem hydraulic vulnerability have been observed in tree species (32). Therefore, we could expect the safety margin to be dynamic in relation with the change in drought exposure. Unexpectedly, we found that stem vulnerability was dynamic over the growing season ($\Psi_{50}$ shifted from $-2.07 \pm 0.08$ to $-2.82 \pm 0.03$ MPa in July and September, respectively; Fig. 3). Furthermore, the $\Psi_{50}$ values observed here were lower than those observed at an earlier stage of plant development (May) reported using HRCT (that is, $<-1.7$ MPa (18)) and were almost identical with the $\Psi_{50}$ values observed in Chardonnay of similar age via nuclear magnetic resonance (31), both noninvasive methods using intact plants. Because plants were continuously well watered from budburst, hydraulic conductivity was stable from June to October ($P = 0.387$), and the mean vessel lengths were shorter than 66 cm, changes in hydraulic vulnerability across the season can only be attributed to ontogenic changes. Increased lignification of stem tissues, along with increased thickness of pit membranes from spring to autumn, likely contributes to this increasing resistance to air seeding (33).

Stems originating from rootstocks appeared slightly but significantly more resistant than those of *V. vinifera* [lower $\Psi_{50}$ ($P < 0.001$) and slope ($P < 0.001$; Fig. 3B)]. However, stem vulnerability did not differ across six different grafted combinations between the scions (Grenache or Syrah) and rootstocks [Selection Oppenheim 4 (SO4), *Vitis riparia* ‘Gloire de Montpellier’ (RGM), or 110 Richter] for $\Psi_{50}$ nor for slope (Fig. S5). Previous studies reported changes in drought tolerance conferred by the identity of the rootstock (34). Because no difference was observed across varieties grafted onto different rootstocks, it is unlikely that these observed differences were brought about by differences in scion stem vulnerability.

**Vineyards maintain a significant hydraulic safety margin**

Unrecoverable water potential (that is, the $\Psi$ below which transpiration cannot be restored to normal values after rewatering ($\Psi_{recov}$)) varies between plant taxa and has been observed at $\Psi_{50}$ in conifers (3) and at higher losses of conductivity in angiosperms ($\Psi_{88}$ (35) or even $\Psi_{99}$ (36)]. We hypothesized that $\Psi_{recov}$ for grapevine would be similar to other angiosperms. Plants exposed to various levels of drought stress during the greenhouse experiment were rewatered to field capacity, and stomatal conductance was monitored at the leaf and plant scale for several weeks. Surprisingly, stomatal conductance of leaves on rewatered plants did not recover to control levels when $\Psi_{pd}$ reached values lower than $\Psi_{50}$ (that is, $\Psi_{recov}$; Fig. 4). However, the $\Psi_{recov}$ was similar across varieties [approximately $-2.76 \pm 0.07$ and $-2.61 \pm 0.08$ MPa (that is, approximately $\Psi_{50}$) at the leaf and whole-plant scale, respectively]. Although some individuals produced new leaves that exhibited normal transpiration, stressed leaves did not recover, most likely because of an increase in leaf abscisic acid concentration (37) and/or petiole and leaf vein embolism. Limited

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transpiration, combined with the hydraulic vulnerability segmentation observed in grapevine petioles, appears to favor leaf shedding under strong and prolonged stress, buffering the perennial organs from reaching lower $\Psi$ (18).

Our greenhouse study demonstrate that when the minimum $\Psi$ leads to 50% loss of stem hydraulic conductivity (that is, $\Psi_{50}$), the vine sheds essentially all of its leaves (leaf mortality, 83.6%; Fig. 5A). Furthermore, under the most extreme conditions observed over approximately a decade in two major cultivation areas, minimal $\Psi_{stem}$ never reached $\Psi_{50}$ [that is, Saint-Emilion and Napa Valley; Fig. 5], although there was severe leaf drop. These data represent a broad range of field conditions and both nonirrigated (Saint-Emilion and Napa Valley) and irrigated (Napa Valley) vineyards. Midsummer is the period with the smallest hydraulic safety margin, and using the thresholds determined in this study, the most extreme drought events would have only generated an ~25% loss in stem hydraulic conductivity (Fig. 5B). Under the normal operating range of $\Psi$ typically observed in Napa Valley in July (from $\Psi_{pd} = -0.36 \pm 0.01$ MPa to $\Psi_{mds} = -1.15 \pm 0.03$ MPa, mean $\pm$ SE), $V. \text{vinifera}$ cv. Syrah would not present significant level of xylem embolism (Fig. 3A), and this is corroborated in intact plants using HRCT (Fig. 6).

Whereas the extent of drought stress can increase along growing season, so does resistance to embolism. Furthermore, $\Psi$ and $g_s$ regulation prevents $\Psi_{stem}$ from reaching these critical levels, and further protection is proved by petiole embolism and leaf mortality. Although extreme seasons surely result in significant leaf mortality and crop loss, more severe drought-induced embolism appears to be uncommon for grapevine. Nevertheless, the midsummer, with its smaller safety margin, likely represents a critical period when considering management under extreme drought.

**CONCLUSIONS**

Here, we define the dynamic hydraulic safety margin for grapevine across the growing season and establish $\Psi$ thresholds critical in increasing viticultural sustainability and sound water management strategies. Stomatal regulation between supposed contrasting varieties was strikingly similar and dynamic across the (an-)isohydric spectrum, representing an anisohydric to isohydric transition as stress increases. Vulnerability to embolism was almost identical between varieties and was not influenced by rootstock. Nevertheless, it is dynamic across the growing season, decreasing as stress increases. When compared to the drought stress experienced over decades in two of the world’s premiere wine regions, severe drought-induced embolism appears to be uncommon for...
grapevine. Because of their perennial nature, vineyards are expected to be productive for decades and require a significant up-front investment. Extreme drought can result in vineyards being fallowed; this was the case for tens of thousands of acres during the recent drought in California, with hugely negative economic consequences. The information elucidated here will aid growers in making more intelligent decisions regarding dry farming, fallowing, and irrigation management.

**MATERIALS AND METHODS**

**Greenhouse experiment**

**Plant material**

Dynamic drydown of four varieties of grapevine [*V. vinifera* cv. Grenache; *V. vinifera* cv. Syrah; *V. rupestris* cv. Martin × iberlandieri cv. Rességuer no. 2, also known as 110 Richter; and *V. riparia* cv. Gloire de Montpellier (38)] was measured in a mini-lysimeter greenhouse platform that measures soil water availability and whole-plant transpiration for each individual plant in real time. Temperature, light, and air humidity were monitored every 20 min in two positions of the greenhouse. Air temperature was maintained below approximately 25°C by the greenhouse cooling system, limiting *D* to approximately 2500 Pa. Cuttings of the year (n = 33 per variety), bearing one flushed bud, were planted into 7-liter pots filled with 1 kg of gravel and 5.5 kg of “Terre de Couhins” (20% clay, 18% silt, and 62% sand). Plants were placed in a greenhouse for 2 months before the experiment started and watered with nutritive solution [0.1 mM NH₄H₂PO₄, 0.187 mM NH₄NO₃, 0.255 mM KNO₃, 0.025 mM MgSO₄, 0.002 mM Fe, and oligo-element (B, Zn, Mn, Cu, and Mo)] to prevent any deficiency during the experiment. Plants were not pruned or damaged during the course of the experiment to avoid air seeding in the xylem. On 16 August 2015, plants were watered to field capacity—watered to saturation at the end of the afternoon and drained overnight. Drought stress was induced on 27 plants per variety by stopping irrigation at three different dates: 26 August on 12 plants per variety, 10 September on 12 plants per variety, and 22 September on 5 plants per variety.

Every 2 to 3 days, *Ψ*ₚᵥ was measured on one basal leaf per plant before any light exposure (between 5:00 a.m. and 7:00 a.m.) on three to five plants per variety and treatment. Between 12:00 p.m. and 2:00 p.m., minimum *Ψ*ₕᵥ (*Ψ*ₚᵥₘᵦᵣ) were measured on the same plants, on fully expanded leaves on which gas exchange was measured (see below). For hydraulic conductivity measurements, *Ψ*ₚᵥₘᵦᵣ values were measured between 12:00 p.m. and 2:00 p.m. on fully expanded leaves, which were bagged in plastic covered with aluminum bags from 7:00 a.m. All *Ψ* values were measured using a Scholander pressure chamber (Precis 2000).

On every sampled leaf, leaf area and length (using a leaf area meter; WinFOLIA 2007b, Regent Instrument) and dry weight (after oven drained overnight). Drought stress was induced on 27 plants per variety by stopping irrigation at three different dates: 26 August on 12 plants per variety, 10 September on 12 plants per variety, and 22 September on 5 plants per variety.

**Gas exchange analysis**

Leaf gas exchange measurements were conducted between 9:00 a.m. and 12:00 a.m. on mature, well-exposed leaves using a portable open-system including an infrared gas analyzer (GFS 3000, WALZ). Conditions in the cuvette were set steady to conditions that were sub-optimal but easily reproducible all along the experimental period (that is, photosynthetically active radiation (PAR), 1500 μmol m⁻² s⁻¹; temperature, 20°C, vapor pressure deficit (*D*), 1 kPa; and CO₂, 400 parts per million).

**Balance data analysis**

All 132 pots were weighed every 15 min for approximately 2 months on individual scales (CH15R11, OHAUS-type CHAMP). Transpiration per leaf area (*E* in mol m⁻² s⁻¹) was estimated as

\[ E = \frac{\Delta w}{A_L} \cdot \operatorname{MM}_w \]  

where *Δw* is the change in weight within the considered period (g s⁻¹), *A*_L is the leaf area (m²), and MMₜₜ is the molar mass of water (18 g mol⁻¹).

Unrealistic values of transpiration in the data set were filtered out. These values originated from plant watering (*Δw* > 0), gas exchange analysis, or other plant manipulations (*Δw* < −100 g hour⁻¹). Low *A*_L (<0.05 m⁴) and low *Δw* (<−2.5 g hour⁻¹; close to balance weight resolution) led to overestimated transpiration rate and were therefore discarded. The whole-plant stomatal conductance *G*ₜ (in mol m⁻² s⁻¹ MPa⁻¹) can be calculated as follows (29).

\[ G_t = K_G(T) \cdot \frac{k_{tot}}{A_L} \cdot \frac{\Psi_t - \Psi_g}{VPD} \]  

where *K*ₕᵥ is the conductance coefficient (in kPa·m³ kg⁻¹) (39), *k*ₜₒₜ is the whole-plant hydraulic conductance (in mol s⁻¹ MPa⁻¹), *Ψ*ₜ and *Ψ*ₜ are the *Ψ* in soil and guard cells, respectively (in megapascals), and D is the vapor pressure deficit (in kilopascals, calculated from temperature and relative humidity data, as indicated in the infrared gas analyzer manual WALZ GFS-3000).

Whole-plant conductance *k*ₜₒₜ was calculated as

\[ k_{tot} = \frac{\Delta w}{\Psi_s - \Psi_g} \cdot \operatorname{MM}_w \]  

Finally, using Eqs. 1 to 3, *G*ₜ was subsequently calculated as

\[ G_t = K_G(T) \cdot \frac{E}{VPD} \]  

On the basis of hourly change in weight (*Δw*) and relations between RWC, *Ψ*ₚᵥ, and *Ψ*ₕᵥ, whole-plant stomatal conductance *G*ₜ was compared to leaf level *g*ₜ, (R² = 0.760; *P* < 0.001; fig. S6).

**Hydraulic conductivity**

When targeted *Ψ*ₚᵥ values were reached, intact plants were brought to the laboratory for hydraulic conductivity measurements. The main stem was cut under water until a 7-cm-long piece was obtained, originating in the middle of the plant. Each cut was alternately performed at basal and apical end, with a 20-s delay between cuttings to relax sap tension within the xylem and prevent air bubble from expanding at the end of the sample (40). Both ends of the sample were refreshed with a clean razor blade and connected to a tubing system. Flow rate of a degassed KCl (10 mM) and CaCl₂ (1 mM) solution was
measured through the sample, from an upper tank to a covered tank placed on a precision electronic balance (0.01-ng resolution; Sartorius). After steady flow was reached (after approximately 30 s to 1 min), mean flow rate was calculated as the average of 10 values measured at 10-s intervals. Flow rate was measured at four different water pressure gradients (ranging from 1 to 3 kPa), according to the procedure described by Torres-Ruiz et al. (41). Hydraulic conductance of the sample \( k_s \) was calculated as the slope of the high flow rate versus the applied pressure, after verification of the linearity of the relation \( (R^2 > 0.99) \). Sample was then flushed at high pressure (140 kPa) for 1 min to remove embolism, and conductance was remeasured \( (k_{max}) \). PLC was calculated as

\[
\text{PLC} = \frac{k_{max} - k_i}{k_{max}}
\]

**Determination of the minimum \( \Psi_{\text{reconv}} \)**

The minimum \( \Psi_{\text{reconv}} \) was determined as described by Brodribb and Cochard (3). During the recovery phase, the time required for \( g_i \) and \( G_i \) to reach 50% of its maximum value \( (t_{1/2}) \) was calculated. Linear regressions were fitted to the inverse of \( t_{1/2} \) versus exposed \( \Psi_{pd} \). The \( \Psi_{\text{reconv}} \), corresponding to the \( \Psi \) inducing a failure of \( g_i \) and \( G_i \) to recover, was assessed by determining the x intercept of the linear regression.

**Long-term \( \Psi \) field monitoring**

Seasonal variations in \( \Psi_{pd} \), \( \Psi_{mdl} \), and \( \Psi_{mds} \) water potentials were measured in \( V. \) vinifera plants growing near Bordeaux [Château Cheval Blanc, Saint-Emilion; Global Positioning System (GPS), 44.92°N, 0.19°W: Saint-Emilion], in California (Napa Valley; GPS, 38.43°N, 122.42°W: Napa Valley) and in Languedoc [Institut National de la Recherche Agronomique (INRA) Pech Rouge experimental unit (Gruissan); GPS, 43.08°N, 3.08°W: Pech Rouge]. In Saint-Emilion, two varieties (Cabernet Franc and Merlot) were monitored over the 2003–2016 period on three blocks with different soil compositions (clay, sand, and gravel). In Napa Valley, three varieties (Cabernet Franc, Cabernet Sauvignon, and Syrah) were monitored over the 2010–2015 period. In Pech Rouge (limestone soil), three varieties (Syrah, Grenache, and Marselan) were monitored in 2005. \( \Psi \) values were measured from June until harvest, every 2 weeks \( (\Psi_{pd} \text{ in Saint-Emilion and Pech Rouge and } \Psi_{mdl} \text{ only in Pech Rouge}) \) or every week \( (\Psi_{mds} \text{ in all locations}) \) according to the procedure detailed below. \( \Psi_{pd} \) values were measured on one basal leaf per plant before any light exposure (between 3:00 a.m. and 5:00 a.m.) on 5 to 10 plants per variety and block. Between 1:00 p.m. and 3:00 p.m., \( \Psi_{mds} \) values were measured on fully expanded leaves from the same plant, which were bagged in plastic covered with aluminum bags at least 1 hour before measurement. All \( \Psi \) values were measured using a Scholander pressure chamber (Saint-Emilion, Precis 2000; Napa Valley, PMS Model-1000; Pech Rouge, Soilmoisture Equipment Corp.)

**In situ flow centrifuge technique**

Xylem vulnerability to embolism was assessed on different varieties with the Cavitron technique, a centrifugation-based technique, as described by Cochard et al. (42). Four-year-old plants were not pruned or damaged during the course of the experiment to avoid air seeding in the xylem. Different varieties were used \( (n = 5 \text{ to } 6 \text{ replicates per variety}) \), either fruit-oriented \( (V. \) vinifera Syrah, Grenache, Cabernet Sauvignon, Merlot and the interspecific hybrid Regent) or rootstock-oriented \( (V. \) riparia Gloire de Montpellier, 110 Richter \( V. \) rupestris \( \times \) berlandieri, and SO4 \( V. \) berlandieri \( \times \) \( V. \) riparia). The basal end of long stems \( (L > 3 \text{ m}) \) was cut and shortened to 1-m length under water. The sample was flushed with air at low pressure (1 bar) to empty vessels open on both ends. Centrifugal force was used to establish negative pressure in the xylem and to provoke drought stress–induced embolism, using a custom-built 1-m-diameter honeycomb rotor mounted on a high-speed centrifuge (DG MEGA). The xylem pressure, \( k_s \) and PLC were determined at various speeds to obtain a vulnerability curve.

In parallel, maximum and mean vessel lengths were measured by the air injection method. Compressed air was delivered to the basal end of a long stem \( (L > 3 \text{ m}) \). Pressure was measured using a manometer and constantly set to 1.8 kPa, whereas air flow was measured using an air flow meter (F111B). The stem was cut shorter until air flowed through the samples (approximately 1 m) and every 10 cm \( (30-\text{ to } 100-\text{cm length}) \) and 5 cm \( (5-\text{ to } 30-\text{cm length}) \). Air flow was measured at each length after stabilization and computed as indicated by Pan et al. (43).

**High-resolution x-ray computed tomography**

Synchrotron-based computed microtomography was used to visualize air- and sap-filled vessels in the main stem of \( V. \) vinifera cv. Syrah. In spring 2015, six well-watered plants were brought to the HRCT beamline [Pression Structure Imagerie par Contraste à Haute Énergie (PSICHE)] at the Source Optimisée de Lumière d’Énergie Intermédiaire (SOLEIL) synchrotron facility and were scanned immediately and after 6 days of drydown. The \( \Psi \) was measured on one leaf, previously wrapped in a plastic bag for 3 hours to provide accurate \( \Psi_{stem} \) values with a Scholander pressure chamber (Precis 2000). The middle part of the main stem was scanned using a high-flux \( (3 \times 10^{11} \text{ photons mm}^{-2}) \), 25-keV monochromatic x-ray beam. The projections were recorded with a Hamamatsu ORCA Flash sCMOS camera equipped with a 250-μm-thick LuAG scintillator. The complete tomographic scan included 1500 projections (50 ms each) for a 180° rotation. Tomographic reconstructions were performed using the PyHST2 software utilizing the Paganin method, resulting in 1536³ 32-bit volumetric images. The final spatial resolution was 3³ μm³ per voxel.

The theoretical specific hydraulic conductivity of a whole cross section \( (K_H) \) was calculated from the Hagen-Poiseuille equation using the individual diameter of sap- and air-filled vessels as

\[
K_H = \sum \frac{\pi \cdot \varnothing^4}{128 \cdot \eta \cdot A_{xyl}} 
\]

with \( K_H \) being the specific theoretical hydraulic conductivity \( (\text{in kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}) \), \( \varnothing \) being the mean Feret diameter of vessels (in meters), \( \eta \) being the viscosity of water \( (1.002 \text{ mPa s at } 20°C) \), and \( A_{xyl} \) being the xylem area of the cross section (in square meters).

The theoretical loss of hydraulic conductivity (PLC) was calculated as

\[
\text{PLC} = 100 \cdot \frac{K_{HA}}{K_{Hmax}}
\]

with \( K_{HA} \) and \( K_{Hmax} \) representing the theoretical hydraulic conductivities of air-filled vessels in initial and cut cross sections, respectively.
Statistical analyses and fit 

Statistical analyses and fit were performed using the R software (www.r-project.org). The slope $\sigma$ of the linear regression between $\Psi_{md}$ and $\Psi_{pd}$ was calculated for different varieties and growing conditions (Fig. 1 and fig. S1). For a given variety, the slope $\sigma$ was calculated across different ranges of $\Psi_{pd}$ that is, from 0 to x MPa (see top inset in Fig. 1 depicting $\sigma$ in relation with x across the whole range). For each 0.1-MPa $\Psi_{pd}$ class, the average of the three absolute $\Psi_{md}$ values was used to calculate the lower limit of the hydroscape, according to the procedure described by Meinzer et al. (10). Accounting for the high variability in $\Psi_{md}$ at high $\Psi_{pd}$ (>0.5 MPa), nonlinear fit was also performed using a composite function

For $\Psi_{pd} > \frac{-b}{2\cdot a}$: $\Psi_{md} = a \cdot \Psi_{pd}^2 + b \cdot \Psi_{pd} + c$  

(8)

For $\Psi_{pd} < \frac{-b}{2\cdot a}$: $\Psi_{md} = \min(a \cdot \Psi_{pd}^2 + b \cdot \Psi_{pd} + c; \Psi_{pd})$  

(9)

To extrapolate $\Psi$ during the greenhouse experiment, $\Psi_{pd}$ was calculated from RWC through Campbell modified by van Genuchten equation (fig. S7) (44)

$$\Psi_{pd} = a \cdot (RWC)^{-b} + \Psi_e$$  

(10)

where $\Psi_e$ is the soil $\Psi$ at the air entry point.

Stomatal conductance, at the leaf ($g_s$) or plant scale ($G_s$), depending on $\Psi_{md}$, was fit according to the sigmoid function

$$g_s = \frac{g_{sm}}{1 + e^{slp \cdot (\Psi - \Psi_{so})}}$$  

(11)

where $g_{sm}$ corresponds to maximal stomatal conductance at $\Psi = 0$, $slp$ is the sensitivity to decreasing $\Psi$, and $g_{so}$ is the $\Psi$ inducing 50% stomatal closure.

Loss of hydraulic conductivity, depending on minimal $\Psi_{stem}$, was fit according to the sigmoid function

$$PLC = \frac{100}{1 + e^{slp \cdot (\Psi - \Psi_{so})}}$$  

(12)

where $slp$ corresponds to the sensitivity to decreasing $\Psi$, and $\Psi_{so}$ is the $\Psi$ inducing 50% loss of conductivity.

Stomatal conductance, at the plant level and under saturating light (PAR > 1500 mmol m$^{-2}$ s$^{-1}$), was fit in relation with current vapor pressure deficit according to logarithmic function (29)

$$G_s = -m \cdot \ln(D) + b$$  

(13)

where $b$ corresponds to maximal stomatal conductance at $D = 1$ kPa (hereafter called $G_{ced}$), and $m \cdot [-dG_s/d\ln(D)]$ is the sensitivity of $G_s$ to $D$ (sens). For isohydric species, sens was shown to be proportional to 60% of $G_{ced}$ across a wide range of species and varying predictably depending on the range of $D$ used in the analysis (29).

The slope sens and the intercept $G_{ced}$ of the linear regression between $G_s$ and $\ln(D)$ were calculated for each variety and level of water stress (Fig. 2). For a given variety, the slope sens, and the intercept $G_{ced}$ were calculated across different ranges of $\Psi_{pd}$. This method used a sliding window mechanism of $G_s/D$ pair values ranked from 0 to the lowest observed $\Psi_{pd}$ value in which sens and $G_{ced}$ were estimated. The window was divided into a fixed number of equal-sized (500) by step increment of 1 (Fig. 2C).

Across varieties, comparisons in the parameters of different correlations (for example, $G_{ced}$ sens, and $\sigma$) were performed using t tests from estimate and SEs as proxy of mean and SEs. Different regressions (that is, linear and nonlinear) of the relation between $\Psi_{md}$ and $\Psi_{pd}$ were compared on the basis of the AIC.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/1/eaao6969/DC1

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fig. S1. $\Psi_{md}$ depending on $\Psi_{pd}$ in different $V. vinifera$ varieties and under environmental conditions.

fig. S2. Correlations between $\Psi_{pd}$ and minimum $\Psi_{md}$ ($\Psi_{min}$) that is, average value of three lowest $\Psi_{md}$ per 0.1 MPa wide class of $\Psi_{pd}$ in different $V. vinifera$ varieties (Grenache and Syrah) and under environmental conditions (field and greenhouse).

fig. S3. Midmorning stomatal conductance measured on individual leaves, depending on predawn leaf water potential $\Psi_{pd}$ in four grapevine varieties ($V. vinifera$ cv. Grenache, blue; $V. vinifera$ cv. Syrah, red; 110 Richter; green; $V. riparia$, pink) during a drydown experiment in greenhouse.

fig. S4. Whole-plant stomatal conductance under saturating light, depending on vapor pressure deficit, in four grapevine varieties.

fig. S5. Percent stem loss of hydraulic conductivity depending on applied pressure in $V. vinifera$ cv. Syrah and Grenache grafted on different rootstocks ($V. riparia$, 504, and 110 Richter) after growth cessation (September), using a dedicated 1-m-diameter Cavitron device (Cavi-1000).

fig. S6. Whole-plant stomatal conductance $G_s$ depending on leaf-scale stomatal conductance $g_s$, measured at the same moment (±1 hour).

fig. S7. $\Psi_{pd}$ depending on RWC in four grapevine varieties ($V. vinifera$ cv. Grenache and Syrah, 110 Richter, and $V. riparia$) during a drydown experiment in a greenhouse.

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7. F. Tardieu, T. Simonneau, Variability among species of stomatal control under fluctuating water stress (Fig. 2). For a given variety, the slope sens, and the intercept $G_{ced}$ were calculated across different ranges of $\Psi_{pd}$. This method used a sliding window mechanism of $G_s/D$ pair values ranked from 0 to the lowest observed $\Psi_{pd}$ value in which sens and $G_{ced}$ were estimated. The window was divided into a fixed number of equal-sized (500) by step increment of 1 (Fig. 2C).


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