Title: Predicting stomatal closure and turgor loss in woody plants using predawn and midday water potential

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Author Contributions
T.K. designed and performed most of the experiments, analyzed the data, and wrote the article together with A.J.M.; N.B. developed the piecewise linear regression model, performed measurements on almond trees, and revised the article; M.I.H helped with measurements of water potential and collected leaf pressure volume curves on walnuts, and revised the article; F.D. helped with measurement of water potential and leaf gas exchange on walnuts; M.K.B. acquired plant material, obtained funding for grapevine research, designed the grapevine experiment, and revised the article; S.G. performed measurement of water potential and leaf gas exchange on grapevines; D.A.K. obtained funding for walnut research, acquired the plant material, helped in experimental design, and revised the article; A.J.M. obtained funding, helped in experimental design and wrote the article together with T.K.

One-sentence summary:
The relationship of plant water potential at predawn and midday exhibits three distinct phases, and the boundaries between phases predict stomatal closure and turgor loss.

Running title:
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Abstract
Knowledge about physiological stress thresholds provides crucial information about plant performance and survival under drought. In this study we report on the triphasic nature of the relationship between plant water potential ($\Psi$) at predawn and midday and describe a method that predicts $\Psi$ at stomatal closure and turgor loss exclusively from this water potential (WP) curve. The method is based on a piecewise linear regression model that was developed to predict the boundaries (termed $\Phi_1$ and $\Phi_2$) separating the three phases of the curve and corresponding slope values. The method was tested for three economically important woody species. For all species, midday $\Psi$ was much more negative than predawn $\Psi$ during phase I (mild drought), reductions in midday $\Psi$ were minor while predawn $\Psi$ continued to decline during phase II (moderate drought), and midday and predawn $\Psi$ reached similar values during phase-III (severe drought). Corresponding measurement of leaf gas exchange indicated that boundary $\Phi_1$ between phase I and II coincided with $\Psi$ at stomatal closure. Data from pressure-volume curves demonstrated that boundary $\Phi_2$ between phase II and III predicted $\Psi$ at leaf turgor loss. The WP curve method described here is an advanced application of the Scholander-type pressure chamber to categorize plant dehydration under drought into three distinct phases and to predict $\Psi$-thresholds of stomatal closure and turgor loss.

Keywords: Drought, transpiration, stomatal conductance, iso-/anisohydric, water relations, woody plants
Introduction

Water stress by drought leads to plant mortality linked to carbon starvation and xylem hydraulic failure (McDowell et al. 2018; Choat et al., 2018). Plant survival under drought is dependent on a successful coordination of physiological responses across multiple organizational levels. This includes efficient stomatal regulation to limit excessive water loss to the atmosphere (Brodribb and Holbrook, 2003; Brodribb and McAdam, 2017), changes in root hydraulic and anatomical properties to minimize water loss to a drying soil and reduce the metabolic cost of soil exploration (Zhu et al., 2010; Barrios-Masias et al., 2015; Cuneo et al., 2016), and osmotic regulation and turgor maintenance on a cellular level to avoid negative effects on growth (Blum, 2017). The exact sequence of these physiological events needs to be elucidated across many species, but data indicate that drought-induced stomatal closure is well correlated with leaf turgor loss and the decline in leaf xylem hydraulic conductance (Brodribb and Holbrook, 2003). Scoffoni et al. (2018) showed that drought-induced changes in cell membrane permeability are linked to stomatal closure and that leaf xylem cavitation is negligible above the turgor loss point. Recent reports suggest that stomatal closure is not triggered by xylem cavitation, but stomatal closure appears to be primarily driven by a decline in root hydraulic conductance and a breakdown of the root-to-soil hydraulic continuum (Carminati et al., 2020; Rodriguez-Dominguez, 2020). For intact plants subjected to progressive drought stress, in-vivo studies indicate that stomatal closure and root cortical cell damage precedes xylem cavitation in the stem, which precedes the discharge of stored water from xylem fibers surrounding vessels (Cuneo et al., 2016; Knipfer et al., 2019a).

Drought-induced stomatal closure plays an important role in minimizing excessive negative pressure in xylem sap ($P_x$) which contributes to increased drought resistance by reducing the risk of xylem cavitation (Martin-StPaul et al., 2017). Although a matter of debate, the isohydry/anisohydry concept provides a framework to describe the efficiency of a plant to control $P_x$ (water potential, $\Psi$, see following paragraph) through stomatal opening/closing under drought (Tardieu and Simonneau, 1998, Martinez-Vilalta et al., 2014, 2017; Ratzmann et al. 2019). As suggested by Meinzer et al. (2016), maintenance of plant $\Psi$ at midday while predawn $\Psi$ declines is indicative for an isohydric behavior while a decline in midday $\Psi$ together with predawn $\Psi$ points to an anisohydric behavior due to inefficient and/or uncoordinated stomatal closure. However, it remains elusive if $\Psi$ thresholds corresponding to stomatal closure and
turgor loss can be predicted directly from measurements of plant $\Psi$, but if so, this would certainly allow for a more time-effective and less-labor intensive assessment of both physiological responses.

The cohesion-tension theory predicts that transpirational pull induces a negative pressure in xylem sap (Dixon and Joly, 1894). The pressure chamber technique allows measuring plant $\Psi$ in a relatively simple way and in turn obtain a good estimate of $P_x$ when leaf apoplast is in equilibrium with symplast prior to leaf pressurization (Scholander, 1965, Turner, 1981). Commonly, the difference between measured $\Psi$ and actual $P_x$ is small since the osmotic potential of the xylem sap is less than 0.1 MPa and the matric potential of the apoplast is close to $\Psi$ of the symplast (Turner, 1981). For walnut (Juglans) trees, Cochard et al. (2001) confirmed that the pressure chamber technique accurately predicts the existence of large negative $P_x$.

Since plants function within a hydraulic continuum between soil and atmosphere, plant $\Psi$ and $P_x$ are dependent on the rate of water loss by transpiration into the atmosphere and water uptake by roots from the soil. Because evaporative demand and light intensity change diurnally and impact transpiration via stomatal regulation, plant $\Psi$ is most negative at midday (i.e. maximum transpiration) and least negative at predawn (i.e. negligible transpiration) especially under well-watered conditions (Klepper, 1968). Plant $\Psi$ at predawn provides an indicator for soil water potential when nighttime transpiration is minimal and plants can reach an equilibrium with the wettest portion of the soil (Turner, 1981; Donovan et al., 2001). On the other hand, progressive drought stress results in a general decline in plant $\Psi$ due to limitations in root water uptake and the fact that evaporative demand is not matched by water supply from the soil. In summary, plant $\Psi$ measurements can provide an integrative measure of plant physiological responses to water stress by drought but the relationship of $\Psi$ at predawn and midday under these conditions is surprisingly understudied.

About 40 years ago, Turner and Long (1980) showed that the relationship of $\Psi$ measured on a covered (non-transpiring) and uncovered (transpiring) leaf is nonlinear for plants subjected to progressive drought stress, which highlighted the variable effect of transpiration on measurements of leaf $\Psi$. Martinez-Vilalta et al. (2014) presented a first theoretical framework regarding the relationship of leaf $\Psi$ measured at midday and predawn, and the authors
interpreted the slope of the relationship as the relative sensitivity of transpiration rate to increasing water stress by drought and the intercept as the maximum transpiration rate per unit hydraulic transport capacity. Similarly, Meinzer et al. (2016) considered the impact of stomatal regulation on plant $\Psi'$ and found that the relationship of $\Psi'$ at midday and predawn (i.e. measured following leaf covering and equilibration) is correlated with a species' turgor loss point and degree of iso-/anisohydricity (i.e. slope of curve and hydroscope). In contrast, Williams and Araujo (2002) reported that the relationship of $\Psi'$ at midday and predawn is linear, but this finding may not hold true under more severe drought and stomatal closure. We revisited these findings for walnut (Juglans), grapevine (Vitis), almond (Prunus dulcis) and performed an in-depth analysis of the water potential (WP) curve between midday and predawn $\Psi$.

Measurements of predawn and midday $\Psi$ were complemented with measurements of leaf gas exchange (stomatal conductance and CO$_2$ assimilation rate) for walnut and grapevine. In addition, for walnut, leaf sap osmotic pressure and pressure-volume curves were collected to obtain an estimate of leaf turgor and $\Psi$ at turgor loss. To test if characteristics of the WP curve are dependent on the type of drought experiment (Gilbert and Medina, 2016), walnut trees were subjected to a slow (weeks) and fast drydown (days, not irrigated). For analysis of the WP curve, a mathematical approach was developed to determine the phases of plant dehydration and to calculate boundaries $-\Theta_1$ and $-\Theta_2$ separating phase-I and -II and -II and -III, respectively. Together, this allowed us to test the hypothesis that calculated $\Theta$-values from the WP curve predict $\Psi'$ at stomatal closure and turgor loss. To avoid confusion, we will not use the existing terminology of ‘$\Psi'_{leaf}$’ and ‘$\Psi'_{stem}$’ since in both cases $\Psi'$ is measured on an excised leaf that is either bagged for a relatively short (<10sec and non-equilibrated) or long (>15min and equilibrated) time period, respectively.

**Results**

**Walnut**

The relationship between water potential measured at predawn ($\Psi_{pd}$) and midday ($\Psi_{md}$) exhibited a nonlinear behavior for walnut trees subjected to the slow drydown (Figure 1A) and fast drydown (Figure 1C). Reductions in predawn and midday $\Psi'$ during the slow and fast drydown were associated with a decline in soil moisture from around 75% to 45% w/w and 80% to 50% w/w, respectively (see Supplemental Figure S1). In general, the relationship between
predawn and midday \( \Psi \) can be described as follows: Early on during drought stress \( \Psi_{md} \) was much more negative than \( \Psi_{pd} \); this was followed by minor reductions in \( \Psi_{md} \) while \( \Psi_{pd} \) continued to decline under moderate drought; \( \Psi_{md} \) and \( \Psi_{pd} \) were most similar under severe drought stress (Figure 1A and 1C).

Based on the smoothed line fit included in Figures 1A and 1C, transition points (\( \gamma \)) along the \( \Psi \)-curve were calculated from \( \frac{d \Psi_{pd}}{d \Psi_{md}} \) slope values (Figure 1B and 1D). Transition points of \( \gamma_1 \) and \( \gamma_2 \) were necessary to parameterize the piecewise linear regression (PLR) model. Subsequently, a statistical estimate for boundary \( \Theta_1 \) between phase-I and -II and boundary \( \Theta_2 \) between phase-II and -III was obtained. The model predicted a boundary \( \Theta_1 \) for the slow and fast drydown of -0.8 MPa and -0.5 MPa, respectively (Figure 2, Table 1). Comparing both drydown experiments, \( \Theta_1 \) was less negative (by around 0.3 MPa) during the fast drydown.

Boundary \( \Theta_2 \) was at -1.3 MPa (slow) and -2.2 MPa (fast), and \( \Theta_2 \) was more negative (by around 0.9 MPa) during the fast drydown (Figure 2). Representative images taken during the slow drydown showed that leaves appeared dark green and turgid for trees in phase-I, leaves lost turgidity and appeared droopy at boundary \( \Theta_2 \) in phase-II, and leaves decolorized to light green and started to desiccate and senesce at the bottom of the canopy in phase-III (Supplemental Figure S2).

The boundary \( \Theta_1 \) between phase-I and -II corresponded to the \( \Psi \)-threshold at which leaf gas exchange was substantially reduced in walnut trees (Figure 3). The different values of \( \Theta_1 \) as determined during the slow and fast drydown with the PLR model reflected the shift in \( \Psi \)-threshold at which stomatal conductance (\( g_s \)) and CO\(_2\) assimilation (\( A \)) reached a minimum. For the slow drydown, \( g_s \) and \( A \) of a mature leaf was reduced by 89% and 86%, respectively, when reaching boundary \( \Theta_1 \) (Figure 3A and 3B). These reductions were similar for the fast drydown (Figure 3C and 3D).

The boundary \( \Theta_2 \) between phase-II and -III corresponded to the \( \Psi \)-threshold that marked the end of the turgor maintenance phase and was indicative of the turgor loss point (\( \Psi_{TLP} = -1.4 \pm 0.14 \) MPa, see Supplemental Table S1) (Figure 4). For the slow drydown, \( P \) initially declined by
around 60% during phase-I, $P$ was maintained during phase-II, and subsequently $P$ declined again during phase-III (Figure 4A). These drought-induced changes in $P$ were associated with an increase in leaf sap osmotic pressure ($\pi$) (Figure 4B). For the fast drydown, $P$ declined predominantly during phase-I (Figure 4C), similar to the slow drydown. However, given the drydown speed, we were not able to collect enough data points of $P$ and $\pi$ to conclusively report on the pattern of data points in phase-II and -III (Figure 4C and 4D).

**Grapevine**

The relationship between $\Psi_{pd}$ and $\Psi_{md}$ was nonlinear (range from 0 to -2.5 MPa) for grapevine plants subjected to a slow drydown (Figure 5). Following initiation of water stress by drought, $\Psi_{md}$ was more negative than $\Psi_{pd}$ (see deviation from 1:1 line). Subsequently, reductions in $\Psi_{md}$ were minor while $\Psi_{pd}$ continued to decline under moderate drought; values of $\Psi_{md}$ and $\Psi_{pd}$ were most comparable under severe drought (Figure 5A). Following the smoothed line fit of predawn and midday $\Psi$ data, the transition points along the $\Psi$-curve were located at $\gamma_1$ of -0.58 MPa and $\gamma_2$ of -1.57 MPa (Figure 5B). Subsequently, the PLR model predicted for grapevine that boundary $\Theta_1$ between phase-I and -II was at -0.68 MPa and boundary $\Theta_2$ between phase-II and -III was at -1.33 MPa (Figure 6, Table 1).

Leaf gas exchange was measured for grapevine (Figure 7). This showed that boundary $\Theta_1$ between phase-I and -II matched the $\Psi$-threshold at which leaf gas exchange was substantially reduced (Figure 7). Following an initial increase of $g_s$ (Figure 7A) and $A$ (Figure 7B), that reached a maximum at $\Psi_{pd}$ of around -0.4 MPa, $g_s$ and $A$ declined by 93% and 67%, respectively, thereafter reaching values of close to zero at boundary $\Theta_1$.

**Almond**

Similar to data collected for potted walnut (see Figure 2) and grapevine (see Figure 6) plants, data collected for almond trees showed that the relationship of $\Psi_{pd}$ and $\Psi_{md}$ exhibited a triphasic curve shape (Figure 8). The calculated boundary values using our statistical method were $\Theta_1 = -1.37$ MPa and $\Theta_2 = -1.94$ MPa (Figure 9, Table 1). See Supplemental Table S2 for statistical comparison of all output parameters obtained with piecewise linear regression (Table 1).
Discussion

In this study, we describe a method that allows predicting stress thresholds associated with drought-induced stomatal closure and turgor loss exclusively from measurements of plant $\Psi$ using a Scholander-type pressure chamber. For woody species (walnut, grapevine, almond), we found that the water potential (WP) curve between plant $\Psi$ at midday and predawn exhibits a unique triphasic curve shape. To quantify the underlying curve parameters, we developed a piecewise linear regression (PLR) model for statistical analysis of the WP curve. For walnut and grapevine, modelling data together with leaf gas exchange data indicated that boundary $\Theta_1$ between phase I- and -II marked the $\Psi$-threshold at which stomatal conductance was reduced by more than 90%. For almond, leaf gas exchange was reduced by approximately 70% at predawn $\Psi$ of -1.4 MPa (Marsal et al., 1997, Prunus dulcis (Mill.) D.A. Webb), and this suggests that our calculated boundary $\Theta_1$ at -1.37 MPa for cultivar 'Nonpareil' most likely predicts stomatal closure as well. Our data of leaf osmotic pressure and $\Psi_{TLP}$ from pressure volume curves indicated that the boundary $\Theta_2$ between phase-II and -III marked the end of the turgor maintenance phase and $\Psi$ at leaf turgor loss for walnut. For grapevine and almond, literature data of $\Psi_{TLP}$ (-1.4 MPa for grapevine 'Chardonnay', Alsina et al, 2007; -2.1 MPa for almond 'Garrigues', Torrecillas et al., 1996) closely matched our calculated $\Theta_2$. The robustness of our WP curve method was tested for walnut by analyzing two types of drydown experiments (slow over weeks versus fast over days and no irrigation). This showed that the WP curve method was successful in identifying the shift in $\Psi$ at stomatal closure depending on the type of drought experiment. Moreover, preliminary data collected for commercially available walnut rootstocks RX1 (Juglans microcarpa X J. regia) and VX211 (J. hindsii X J. regia) indicated that WP curves generated from the relationship of predawn and midday $\Psi$ are genotype-specific (Supplemental Figure S3). In summary, the WP curve method presented here provides for a new approach to evaluate plant drought responses (stomatal closure, turgor loss) in a cost-effective and relatively simply way that only requires access to a Scholander-type pressure chamber.

Drought-induced stomatal closure

In the past, Turner and Long (1980) reported on the nonlinear behavior of the relationship of plant $\Psi$ measured simultaneously on a covered leaf prior to excision and an uncovered leaf.
Their data show a triphasic curve shape with values approaching 1:1 due to stomatal closure when plants experience severe drought. Similarly, our data demonstrate that the relationship of plant $\Psi$ measured at predawn and midday is nonlinear and triphasic. Our leaf gas exchange measurements indicated that stomatal conductance ($g_s$) reached a minimum at the transition point between phase-I and -II (i.e. boundary $\Theta_1$) of the WP curve which confirmed that stomatal closure was the main driver that caused the initial change in shape of the WP curve. We speculate that the shape of the curve between $g_s$ and predawn $\Psi$ until reaching boundary $\Theta_1$ is dependent on how well drought-induced closure between individual stomates of a leaf is coordinated. For example, a distinctive drop-off in measured $g_s$ should only be observed at boundary $\Theta_1$ when all stomates transition instantaneously from an open to a closed state (see Gollan et al., 1985). Following this logic, we hypothesize that the shape of the WP curve during phase-I is predominantly affected by the ability of a plant to efficiently coordinate stomatal closure under increasing water stress by drought.

Stomatal closure/opening can be triggered by several factors including light intensity, vapor pressure deficit (VPD), abscisic acid (ABA) concentration and/or hydrostatic pressure (Farquhar and Sharkey 1982; Franks 2013; Tombesi et al., 2015). We did not measure ABA concentrations, but our knowledge about the role of ABA in stomatal closure can be summarized as follows: A root tip that is exposed to dry soil conditions commonly synthesizes additional amounts of ABA which ultimately arrives in leaves depending on the transport efficiency of the transpiration stream (Zhang et al., 1987). Gollan et al. (1986) showed that when leaves are kept turgid by pressurizing roots while the soil is drying, stomates still closed, which was interpreted as further evidence for the importance of root-to-shoot signaling via ABA translocation. However, McAdam and Brodribb (2018) found that leaf mesophyll cells are the main location for ABA biosynthesis under drought stress which de-emphasizes the importance of ABA delivery from roots to leaves via the xylem to initiate stomatal closure. Drought-induced increases in root ABA concentrations may also trigger aquaporin-mediated changes in root hydraulic conductivity and/or result in modifications of root architecture (Maurel et al., 2008; Rosales et al. 2019). Recent studies suggest that drought-induced changes in root hydraulic properties drive stomatal closure (Carminati et al., 2020; Rodriguez-Dominguez and Brodribb, 2020). Besides ABA, drought-induced stomatal closure can be mediated by a pressure-induced passive mechanism depending on xylem pressure (Brodribb and McAdam, 2011; Franks, 2013;). Tombesi et al. (2015) reported that $g_s$ in grapevines subjected to drought stress is
predominantly regulated by such a passive hydraulic signal. The authors came to this conclusion because leaf ABA increased only after complete stomatal closure, and it was hypothesized that this is of importance for long-term drought recovery to facilitate xylem embolism repair by forcing stomatal closure when the soil is rehydrated. In summary, and in the context of collecting WP curves for various woody plants, we speculate that if leaf ABA accumulation is observed in phase-I, this points to a predominant ABA-mediated mechanism driving stomatal closure, whereas if leaf ABA accumulation is observed in phase-II this points to a predominant pressure-driven (passive) mechanism driving stomatal closure.

Atmospheric evaporative demand affects the rate of transpiration (see introduction; Klepper, 1968). Gollan et al. (1985) performed an extensive study on the relationship of vapor-pressure-deficit (VPD), $\Psi$ determined on leaves with in-situ psychrometers and soil water content on leaf gas exchange. The authors showed that the relationship of $\Psi$ and leaf gas exchange is dependent on VPD. During increasing drought, their data point to a sharp drop-off in $g_s$ at high VPD (25 Pa kPa$^{-1}$) whereas the reduction in $g_s$ was gradual until reaching a minimum (as observed in this study) at low VPD (10 Pa kPa$^{-1}$). Due to natural fluctuations in VPD and its effect on transpiration, plant $\Psi$ can vary between days and over the course of a day for the same soil water status. For this reason, plant $\Psi$ at midday (as measured on an equilibrated leaf using a pressure chamber, i.e. ‘$\Psi_{stem}$’) is most meaningful as a water stress indicator over the growing season when compared to baseline values under well-irrigated conditions (Turner NC, 1990; Shackel et al., 1997). Based on our findings, we hypothesize that effects of VPD on the shape of the WP curve are most pronounced during phase-I prior to stomatal closure, and high versus low VPD conditions result in either a steeper (higher transpiration) or shallower (lower transpiration) slope $\beta_1$. On the other hand, and for a given plant species, we speculate that the effect of VPD on boundary $\Theta_1$ is negligible if stomatal closure is predominantly driven by soil-to-root interactions (see previous paragraph).

Leaf turgor loss

Our data provide evidence that boundary $\Theta_2$ separating phases-II and -III of the WP curve predicts $\Psi_{TLP}$. For walnut (variety ‘Cisco’), average $\Psi_{TLP}$ determined from pressure-volume (PV) curves of -1.39 MPa (Supplemental Table S1) was only slightly more negative as compared to
For grapevine (variety ‘Chardonnay’), Alsina et al. (2007) reported an average $\Psi_{\text{TLP}}$ of -1.36 MPa at veraison and prior berry maturation, which was similar to our $\Theta_2$ of -1.33 MPa (Table 1). For almond, Torrecillas et al. (1996) determined a $\Psi_{\text{TLP}}$ of around -2.2 MPa (cultivar ‘Garrigues’) and -2.3 MPa (cultivar ‘Ramilete’) for well-watered trees as compared to our $\Theta_2$ of -1.9 MPa (cultivar ‘Nonpareil’) (Table 1). Together, this suggests that $\Psi_{\text{TLP}}$ can be predicted from the triphasic WP curve using our PLR model for a variety of woody species.

For walnut trees subjected to the slow drydown, we were able to collect sufficient data points to elucidate the pattern of drought-induced variations in leaf turgor (i.e. $P = \Psi - \pi \sigma$; Jones and Turner, 1978). To calculate $P$, we assumed that $\pi \approx \pi_{\text{symplast}}$ and that the reflection coefficient for solutes ($\sigma$) was unity. If we consider the remaining apoplastic sap in the centrifuged leaf tissue resulted in a dilution effect (Wardlaw, 2005), then our measured $\pi$ underestimated true $\pi_{\text{symplast}}$. Hence, true $P$ should be slightly lower than our calculated $P$, which would explain why our calculated $P$ was 0.3 MPa and not 0 MPa at $\Theta_2$ of -1.31 MPa (see Figure 4A). On the other hand, $\pi_o$ at full hydration obtained from PV curves was 1.1 MPa (Supplemental Table S1) but $\pi$ at predawn $\Psi$ of 0 MPa was around 1.7 MPa (Figure 4A), which points to a possible overestimation of $\pi_{\text{symplast}}$. The indirect approach used here provides a relatively easy way to obtain information on drought-induced $P$ changes, but if experimentally feasible, $P$ and $\pi_{\text{symplast}}$ are best determined directly using a combination of cell pressure probing and picolitre osmometry (Tomos and Leigh, 1999; Fricke and Peters, 2002; Knipfer et al., 2014).

Although controversial, it has been reported that negative turgor exists in plant cells according to indirect measurements from PV curves (Tyree, 1976, 1979; Rhizopoulou, 1997, Ding et al., 2014). Our $P$ data obtained indirectly by $\Psi - \pi \sigma$ also point to the existence of negative $P$ which occurred in phase-III following the turgor loss point. However, one factor that may explain the measurement of negative turgor when determined indirectly is $\sigma$, which provides a measure of solute permeability/leakage of the cell membrane (Staverman, 1951; Knipfer et al., 2014). Drought stress results in increased solute leakage and modulation of the physical state of the membrane (Blum and Ebercon, 1981; Premachandra and Shimada, 1987; Couchod et al., 2019). If solute leakage plays a role during drought stress, then $P$ is best determined by $\Psi - \pi \sigma_i$ for $i=\text{level of water stress}$. Hence, if we imagine that solute leakage becomes more and more
severe under increasing drought stress, $\sigma$ would become smaller and smaller and the term $\sigma \pi$ goes towards zero, which in turn would result in a calculated $P$ that may not reach negative values. Comparing the slow versus fast drydown, our data suggest that $P$ reaches values closer to zero during the fast drydown at boundary $\Theta_1$. This may be due to generally higher $\pi$ values in mature leaves of these trees because of seasonal effects (i.e. this drydown was performed in September versus June/July) or less severe cell membrane damage and electrolyte leakage during phase-I when the drydown is fast (i.e. this drydown was performed over a couple of days versus weeks). However, the biophysical properties of the cell membrane under various levels of drought stress and in response to the type of the drought experiment remain unknown, and only direct $P$ measurements using a cell pressure probe would allow us to resolve these open questions (see previous paragraph).

Pressure-volume curve

The Scholander-type pressure chamber has been used successfully to generate pressure-volume (PV) curves for determination of tissue properties such as $\Psi_{\text{TLP}}$, bulk modulus of elasticity, and $\pi_0$ at full hydration (Tyree and Hammel, 1972, Ding et al. 2014). The advantage of the PV curve is that $\Psi_{\text{TLP}}$ can be determined from a single leaf measurement. The disadvantage is that generating a PV curve can be time-consuming (>10h) depending on the speed of leaf dehydration and requires accessibility to an analytical digital balance to determine relative water content; one technical difficulty is finding the right time interval for progressive leaf dehydration and data collection.

Water-potential curve

The WP curve method allows predicting $\Psi$ at stomatal closure and $\Psi_{\text{TLP}}$ exclusively from measurements of $\Psi$ at predawn and midday using a Scholander-type pressure chamber. This can be especially useful under remote field conditions or during research operations with limited access to a leaf gas exchange system, analytical balance, and laboratory space. Moreover, the WP curve method will provide for a time-integrative measurement of $\Psi$ at stomatal closure and $\Psi_{\text{TLP}}$ when plant $\Psi$ at predawn and midday is collected on mature leaves over the growing season. In this case, it is recommended that plant $\Psi$ is measured during the phenological timeframe following leaf maturation and prior senescence to ensure that leaf cells, xylem and...
cuticle are fully developed. For establishing the WP curve, we advocate for the following stepwise procedure for measurement of plant $\psi$ (Figure 10):

1. **Covering of leaf to minimize transpiration**: Identify a representative mature leaf in the canopy and cover the leaf with a plastic bag. Seal plastic bag to allow for a humid environment that aids stomatal closure and minimizes transpiration (Turner and Long, 1980).

2. **Equilibration of leaf apoplast and symplast**: Wrap sealed plastic bag with aluminum foil to exclude ambient light from leaf surface. This will further aid stomatal closure. Wait for more than 30 min to allow for equilibration of leaf internal water potentials of apoplast (liquid to the outside of the cellular membrane including xylem liquid) and symplast (liquid to the inside of the cellular membrane). Including the equilibration will ultimately provide a measure of plant $\psi$ that closely reflects $P_x$ (see introduction).

3. **Excision of leaf**: Cut leaf at petiole (or petiolule) end using scissors or razor blade.

4. **Measurement of plant water potential**: Insert covered leaf into pressure chamber with the cut surface protruding through the seal of chamber lid. Slowly raise pressure in chamber while monitoring the cut surface; use a magnifying glass and a light source if it is difficult to identify xylem vessels. Record the pressure when liquid starts to emerge from open xylem vessels and a meniscus forms on the cut surface.

One alternative procedure to save time is to skip the equilibration step and immediately proceed from step 1 to 3 (Figure 10). However, excluding the equilibration step 2 provides a less accurate estimate of $P_x$ since leaf internal water potential gradients between apoplast and symplast are not minimized prior to leaf excision (Shackel et al., 1997). Another alternative procedure is to seal the excised leaf in a second plastic bag (i.e. minimize leaf water loss as much as possible) and store the sample at around $4^\circ$C for up to 24 hours prior to measurement of $\psi$ (Figure 10); the assumption is that leaf internal water potentials are maintained constant during the storage period, but this should ideally be tested first on a subset of plants through frequent $\psi$ measurements during the storage period.

Our data show that, opposite to a fast drydown, a controlled and slow drydown (weeks) has the advantage of collecting a higher number of data points during all three phases of the WP curve because predawn $\psi$ can be determined for a wide range of soil moisture contents. When establishing the WP curve, predawn $\psi$ can be interpreted as a measurement of soil $\psi$ when
nighttime water loss by transpiration is negligible for the plants analyzed. For plant species that exhibit relatively high rates of nighttime transpiration, the required equilibrium between predawn \( \Psi \) and soil \( \Psi \) cannot be reached (Donovan et al., 2001). Donovan et al. (2001) came to this conclusion by comparing measurements of soil \( \Psi \) with predawn \( \Psi \) as obtained from plants with completely bagged and non-bagged canopy during the night period. For walnut trees subjected to the slow drydown, nighttime canopy conductance was >100-fold smaller compared to daytime values (Supplemental Figure S4, inset) and we can conclude that our measurements of predawn \( \Psi \) closely reflected soil \( \Psi \) for this type of experiment. For every soil type, soil \( \Psi \) can be interpreted as the ability of a plant to extract water from the soil (Jones, 2007). However, the breakdown of the soil-to-root hydraulic continuum is suspected to be the trigger for stomatal closure, not soil \( \Psi \) (Carminati et al., 2000). In turn, it can be speculated that boundary \( \Theta_1 \) also provides an estimate when the hydraulic continuum between root and soil starts to break down under increasing water stress by drought independent of the soil type.

**Type of drought experiment**

Limited soil water availability is the factor that causes drought stress. Plant stress responses to drought depend on drought severity and a plant’s ability to adapt to the stress over time (Gilbert and Medina, 2016). Therefore, the experimental procedure of how drought is induced over time (i.e. days versus weeks) can result in a more or less severe stress response for a given level of soil moisture. A slow drydown (weeks) would provide time for stress adaptions linked to plant anatomical changes, for example, root suberization to minimize water loss back to the soil and reductions in vessel diameters to secure long-distance transport capacity (Barrios-Masias et al., 2015; Knipfer et al. 2015, 2020). Here, we conducted a slow and fast drydown experiment for walnut trees to test if the WP curve (i) remains triphasic and (ii) predicts stomatal closure in both types of experiments. Our data show that the WP curve was triphasic in both drydown experiments but the character of the WP curve differed between the slow and fast drydown and boundary values \( \Theta_1 \) and \( \Theta_2 \) were specific to the drydown experiment (see Table 1). Boundary \( \Theta_1 \) predicted the shift in \( \Psi \)-threshold of stomatal closure in both types of drydown experiments. We speculate that boundary \( \Theta_1 \) was less negative during the fast drydown, i.e. earlier stomatal closure, because the time period for anatomical adaptations - which would aid to maintain plant performance for the imposed level of stress - was too short. Data by Knipfer et al. (2020) show that walnut fine roots develop a multi-seriate endodermis in response to a slow drydown, and
data by Meyer et al. (2009) indicate that the development of a multiseriate exodermis in *Iris germanica* requires approximately 12 days. However, and especially for the purpose of using the WP curve method to select for genotypes with improved drought resistance, future work is required to exactly determine the effect experimental time period, soil medium, irrigation frequency and pot characteristics on the shape of the WP curve (Turner, 2019).

### Conclusion and future implications

The WP curve methods represents a methodological advancement of the Scholander-type pressure chamber to predict $\Psi$ at stomatal closure and turgor loss. Based exclusively on measurements of plant $\Psi$ at predawn and midday, the method presented here provides an alternative tool for the study of plant stress physiology in natural and agricultural ecosystems. According to published data for grapevine and under the assumption that $\Psi$ reported by these authors (i.e. measured under laboratory low light conditions) is most representative of predawn $\Psi$ (Choat et al., 2010; Brodersen et al., 2013 Cuneo et al., 2016), the following can be concluded: i) root cell damage is initiated at boundary $\Theta_1$ at a $\Psi$-threshold that corresponds to stomatal closure, ii) root hydraulic conductivity declines progressively during phase-I and -II and reaches a minimum at boundary $\Theta_2$ at a $\Psi$-threshold that corresponds to leaf turgor loss, iii) vessel cavitation is initiated at boundary $\Theta_2$ and cavitated vessels accumulate during phase-III. This example demonstrates that our WP method can be used to categorize the sequence of physiological and anatomical events that occur under progressive drought stress into three distinct phases. Moreover, we propose that the WP curve method can assist in the determination of $\Psi$-thresholds that mark the breakdown of the soil-to-root hydraulic continuum (Rodrigues-Dominguez and Brodribb 2020; Carminati et al., 2020) and xylem hydraulic failure by embolism (Cuneo et al., 2016; Knipfer et al., 2019;), and facilitate the selection for woody perennial genotypes with improved drought-resistance (Knipfer et al., 2020).

### Materials and methods

#### Piecewise linear regression (PLR) model

Initial data inspection showed that a simple linear model did not appropriately describe the relationship of plant water potential ($\Psi$) at predawn (subscript 'pd') and midday (subscript 'md')
for three woody species (walnut, grapevine, almond). For this reason, the PLR model was developed for analysis of the water potential (WP) curve:

\[
\Psi_{md}(\Psi_{pd}) = \begin{cases} 
\alpha + \beta_2 \Psi_{pd}, & \Psi_{pd} \leq \Theta_2 \\
\alpha + \Theta_2 (\beta_3 - \beta_2) + \beta_2 \Psi_{pd}, \quad \Theta_1 \geq \Psi_{pd} > \Theta_2 \\
\alpha + \Theta_2 (\beta_3 - \beta_2) + \Theta_1 (\beta_2 - \beta_1) + \beta_1 \Psi_{pd}, \quad \Psi_{pd} > \Theta_1
\end{cases}
\] (Eq.1)

The PLR model was used to calculate the boundaries between linear phases (\(\Theta_1 = \text{phase-I to -II}\), \(\Theta_2 = \text{phase-II to -III}\)) and corresponding slope values (\(\beta_1 = \text{phase-I}\), \(\beta_2 = \text{phase-II}\), \(\beta_3 = \text{phase-III}\); \(\alpha = \text{intercept}\)) (Eq. 1).

To parameterize the PLR model, an estimate of the transition points between phase-I and -II (\(\gamma_1\)) and phase-II and -III (\(\gamma_2\)) was obtained mathematically as follows. First, a smoothed line was fitted to the relationship of \(\Psi_{pd}\) and \(\Psi_{md}\) that best described the data pattern. Subsequently, slope values (\(m\)) of the smoothed line were determined by \(d\Psi_{md}/d\Psi_{pd}\) for \(d=0.01\) MPa, and maximum \(m\) in phase-I (=\(a\)) and -III (=\(c\)) and minimum \(m\) in phase-II (=\(b\)) were identified. Parameters of \(a\), \(b\) and \(c\) were used to determine \(\gamma_1 = (b-a)/2 + b\) and \(\gamma_2 = (c-b)/2 + c\).

Based on information of \(\gamma_1\) and \(\gamma_2\), all parameters for the PLR model were fitted using a least squares solver implemented in Python (pwfl package version 1.1.6; Jekel and Venter, 2019). This allowed for fitting a continuous piecewise linear function to corresponding data of \(\Psi_{pd}\) and \(\Psi_{md}\) for a specified number of three line segments. Our method used a limited memory Broyden-Fletcher-Goldfarb-Shanno (LM-BFGS) algorithm for bound constrained optimization to obtain a statistical solution of boundary values \(\Theta_1\) and \(\Theta_2\) from the initial estimates of \(\gamma_1\) and \(\gamma_2\). Standard errors and p-values corresponding to output parameters of the PLR model were the result of using this optimization procedure to find boundaries \(\Theta_1\) and \(\Theta_2\) that best satisfied the specified number of linear segments; standard errors were obtained following the derivation of Coppe et al (2011) for linear regression problems.

Plant materials
The WP curve method was tested initially for data collected during a drought screening trial on potted walnut (Juglans) trees (lathhouse trial in 2019, experimental leader Dr. Knipfer). Subsequently, the method was further evaluated by re-analyzing data that were collected during drought screening trials of potted grapevines (Vitis; greenhouse trial in 2019, experimental leader Dr. Bartlett) and potted almond (Prunus dulcis) trees (field trial in 2014, experimental leader Dr. Bambach) at University of California, Davis (USA). Therefore, growing conditions and experimental procedures for data collection for the three woody species were not identical and are best summarized as follows based on the information available:

**Walnut experiments**

Experiments were performed in 2019 on n=71 walnut trees (variety 'Cisco'). Trees (stem diameter and height of around 2.5cm and 1m, respectively) were obtained from Sierra Gold Nursery (Yuba City, CA, USA) and transplanted into 15-liter plastic pots on February and 17th, 2019. Pots were filled with a similar amount of soil mix (approximately 50% washed sand and 50% sphagnum peat moss) by leaving a gap of around 2-cm to the upper edge of the pot. A slow-release fertilizer (Osmocote Smart-Release Plus) was added to the topsoil layer. Growth was maintained under ambient atmospheric conditions (day/night length was on average 14/10h and temperature was ~13/32°C) in a lathhouse (University of California Davis, Plant Pathology, Old Davis Road). Trees were irrigated by supplying water to the top of the soil every two days and maintained well-watered for 3 months after transplanting to ensure sufficient time for tree establishment. All physiological measurements were performed on mature leaves of current-year shoots. A temperature and relative humidity sensor (HMP50; Vaisala, Woburn, MA) was installed at the plot site to monitor vapour-pressure deficit (VPD, see Supplemental Figure S5). To investigate possible effects of type of drought experiment, trees were subjected to a slow drydown (i.e. weeks, adjustment of irrigation) or fast drydown (i.e. days, no supplemental irrigation): The slow drydown experiment was performed on n=65 trees. Irrigation was adjusted based on estimates of bulk soil moisture (SM, = weight\text{H}_2\text{O}/weight\text{H}_2\text{O-pot-capacity} \times 100\% ) as calculated from pot weights (for details see Knipfer et al., 2020). A subset of n=27 plants was located on mini-weighing lysimeters to continuously monitor pot weight and SM (see Supplemental Figure S6 for representative data of two individuals); to account for temporal effects, trees were either maintained well-watered or subjected to a drydown. At 103 days after transplanting, measurements were performed on n=27 trees with SM of individuals ranging from 40 to 90\% w/w (VPD ranged from 2.1 to 2.9 kPa between 11AM and 1PM). At 150 days after
transplanting, measurements were carried out on \( n = 36 \) trees with \( SM \) ranging from 58 to 87% w/w (VPD ranged from 1.9 to 2.5 kPa). At 162 days after transplanting, measurements were performed on \( n = 30 \) trees with \( SM \) ranging from 44 to 75% w/w (VPD ranged from 1.7 to 2.2 kPa). The fast drydown experiment was performed on \( n = 6 \) trees that were maintained well-watered until the start of the drydown. Supplemental irrigation was stopped at 210 days after transplanting. Trees were analyzed at 210 (\( SM \) ranging from 73 to 100% w/w), 213 (69% to 96% w/w), 216 (54% to 86% w/w), 218 (49% to 77% w/w) and 220 (47% to 72% w/w) days after transplanting.

**Water potential** - A pressure chamber (PMS Instrument Company, Model 1505D, Albany, Oregon, USA) was used to measure plant \( \Psi \) following leaf covering and equilibration (i.e. \( \Psi_{stem} \)). Measurements were performed on a leaflet of a mature leaf that was covered with aluminum foil and equilibrated for >1h using a sealed plastic bag. Following excision of the leaflet at the petiolule, the plastic bag was removed, and leaflets still covered with foil (i.e. to exclude effects of transpiration; Turner and Jones, 1980) were inserted into the pressure chamber. The pressure in the chamber was raised slowly at a constant rate (about 0.01 MPa per seconds) and pressure was recorded when a water meniscus started to form on the cut petiolule (midvein of leaflet) surface. For the same plant, \( \Psi \) at predawn was measured prior to sunrise between 4 AM to 6 AM prior to sunrise and \( \Psi \) at midday was measured between 11 AM to 1 PM pacific daylight time. Watering was always completed the day before measurement of \( \Psi \) to allow for soil water distribution.

**Leaf gas exchange** - Stomatal conductance (\( g_{s} \)) and CO\(_{2}\) assimilation rate (\( A \)) were measured between 11 AM and 1 PM using a LICOR-6800 gas exchange system (fan speed at 10.000 rpm, leaf temperature at 24.5°C, CO\(_{2}\) sample at 400 ppm, and 1500 \( \mu\)mol m\(^{-2}\) s\(^{-1}\) light intensity). One non-shaded leaflet of a mature leaf was measured on each sapling that was in proximity to the leaflet used for measurements of \( \Psi \) (as described above). Since the leaf area inserted in the cuvette of gas exchange system during measurement occupies many stomata and the response of individual stomates within this area can be more or less coordinated and homogenous, the point of stomatal closure was defined as the point where \( g_{s} \) and \( A \) reach a minimum.
Leaf turgor and osmotic pressure - From the same leaf used for $\Psi$ and leaf gas exchange, a leaflet was excised with scissors. Immediately after the leaf lamina was separated from the midvein using a razor blade (i.e. to minimize the contribution of apoplastic xylem sap), the leaf lamina (a 2-cm long portion located halfway along the leaflet) was placed in a 2 ml Eppendorf tube containing a mesh filter and stored on ice. Great care was taken to ensure that the procedure, from initial leaflet excision to storage of the Eppendorf tube on ice, was completed within 30 seconds to minimize drying artifacts. All samples were kept on ice, transported to the laboratory within 1 h following sampling, and stored at -80°C until further analysis. For analysis of leaf sap osmotic pressure ($\pi$), samples were thawed at room temperature for 15 to 20 min, centrifuged at 8000 rpm for 15 min to extract leaf sap, and osmolality of leaf sap was measured using a Vapor pressure osmometer (VAPRO 5600, Wescor Inc.) (Barrios-Masias et al., 2018). Measured values of sap osmolality (in mOsmol kg$^{-1}$) were converted to unit osmotic pressure ($\pi$; 0.1 MPa = 40.75 mOsmol kg$^{-1}$). Leaf turgor ($P$) was estimated by $\Psi - \pi$ according to Jones and Turner (1978). Turgor loss point ($\Psi_{TLP}$) was determined from pressure volume curves measured on a leaflet. Prior to measurements, one mature leaf per plant was excised, the cut end of the petiole was submerged in water, the leaf was covered with a plastic bag, and transported to the lab within 30 min. Subsequently, the leaf was allowed to rehydrate in the darkness for 24 h. A leaflet was excised from the compound leaf and turgor loss point was measured using the benchtop drydown method as described by Sack and Pasquet-Kok (2011). Leaflet fresh weight was measured with a digital balance before and after each water potential step measurement (PMS Instrument Company, Model 1505D; Albany, Oregon, USA). Measurements were repeated until five measurements after the turgor loss point ($\Psi_{TLP}$).

Grapevine experiments

Experiments were performed in 2019 on 48 grafted vines (variety Chardonnay on rootstock 5C, 420A, Riparia Gloire, 101-14, Ramsey, 140Ru, 1103P, and 110R). Vines were planted in 6-liter pots. Pots were filled with similar amounts of soil mix (approximately 75% coconut coir and 25% perlite) by leaving a gap of around 2 cm to the upper edge of the pot. Growth was maintained in a greenhouse on the UC Davis campus. Vines were allowed to establish for three months prior to data collection. During the establishment period, the vines were pruned to a single shoot, which was staked and tied after reaching approximately 0.5 m in length. The pots were weighed and irrigated by supplying water to the top of the soil three times per week to a target weight...
(‘pot weight at saturation’ + ‘half of pot evapotranspiration’; evapotranspiration was the difference in pot weights). On September 9th, half of the vines were randomly assigned to each of two watering treatments. The well-watered vines continued to receive the same watering regime as during the establishment period, while the water-stressed vines were watered to 40% of the saturated pot weight plus half of pot evapotranspiration. Water was withheld from water-stressed vines until the pots reached the target weight. Three vines per rootstock \times treatment combination (n = 48) were assessed for gas exchange and pre-dawn and midday leaf water potentials on November 4th and 12th. On December 9th, supplemental watering was stopped for one vine per rootstock from the water-stressed treatment, to allow for severe drought conditions.

Water potential – A mature leaf was covered with a plastic bag just prior to excision from the stem using a razor blade (midday between 1PM to 3PM and predawn between 4AM to 6AM) (i.e. ‘Ψ_leaf’). Subsequently, the bagged leaf was inserted in a sealed plastic bag and stored at 4°C. Leaves were transported to the laboratory, and Ψ was measured within 24 hours following leaf excision using a pressure chamber following the procedure as described for the walnut experiment. Watering was always completed the day before measurement of Ψ to allow for soil water distribution.

Leaf gas exchange – Measurements were performed for one mature, fully-expanded leaf per vine that was in proximity to the leaf used for Ψ measurements. The sampled leaves were selected from exterior, sunlit canopy positions. The gas exchange measurements were conducted at the same time as the water potentials, using a LICOR-6800 at a constant fan speed (10,000 rpm), sample chamber VPD (1.5 kPa), CO₂ concentration (400 ppm), and light intensity (1000 µmol m⁻² s⁻¹). Leaf temperature ranged from 26.9 to 27.5°C.

Almond experiments

Experiments were performed on n=16 potted 4-year-old almond trees (cultivar ‘Nonpareil’). Pots (56 liter) were filled with similar amounts of soil mix (approximately 60% plaster sand and 40% peat moss). Slow-release fertilizer spikes (Miracle-Gro Fruit Fertilizer) were inserted in each pot.
Potted trees were maintained in an open field at the Orchard Park Greenhouse Complex at UC Davis campus. Throughout the experimental period the daily maximum temperature ranged from 19.4 to 50.6 °C. Over the experimental period, trees were watered by supplying water to the top of the soil either once every other day (n=8 trees) or twice daily (n=8 trees).

Water potential - Three mature leaves near the trunk were selected from each tree to perform predawn (5 AM to 6 AM) and midday (12 PM to 1 PM) Ψ measurements with a pressure chamber (PMS Instrument Company, Model 1000, Albany, Oregon, USA). Leaves were covered with an aluminum foil bag and equilibrated for 15 min (predawn) and 45 min (midday) (i.e. ‘Ψ_{stem}’). Following excision, bagged leaves were sealed and transported in a cooler (4°C) to the laboratory and Ψ measurements with the pressure chamber (as described for the walnut experiment) were performed within 1hr after leaf excision. Watering was always completed the day before measurement of Ψ to allow for soil water distribution.

Data analysis
Graphs were generated using SigmaPlot (version 8.0, Systat Software Inc. San Jose, CA, USA). Smoothed lines that best described the pattern of data points were generated using the ‘PROC TRANSREG’ (sm = smoothing parameter) procedure in SAS (version 9.2, SAS Institute, Cary, NC, USA). A statistical comparison of model output parameters was performed using a Z-test (see Supplemental Table S2).

Supplemental Data
Figure S1: Recordings of pot weight and bulk soil moisture for walnut trees.
Figure S2: Representative images of the canopy of walnut trees.
Figure S3: Water potential curve collected for walnut genotypes RX1 and VX211.
Figure S4: Relationship between Ψ and canopy conductance of walnut trees.
Figure S5: Recordings of vapor pressure deficit for walnut trees.

Figure S6: Relationship of predawn $\Psi$ and bulk soil moisture of walnut trees.

Table S1: Turgor loss point determined from pressure-volume curves for walnut trees.

Table S2: Z-test used for statistical comparison of output parameters shown in Table 1.

Acknowledgements

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Table 1: Summary of output parameters from the piecewise linear regression model as used for analysis of the relationship between predawn and midday $\Psi$ for woody species (walnut variety 'Cisco', grapevine variety 'Chardonnay', almond variety 'Nonpareil'). N= number of observations, $\Theta_1$= boundary phase-I to -II, $\Theta_2$= boundary phase-II to -III, $\beta_1$= slope phase-I, $\beta_2$= slope phase-II, $\beta_3$= slope phase-III, $\alpha$ = intercept, $R^2$ = coefficient of determination.

| Parameter | Value | SE  | P    | Value | SE  | P    | Value | SE  | P    | Value | SE  | P    |
|-----------|-------|-----|------|-------|-----|------|-------|-----|------|-------|-----|------|
| Walnut    |       |     |      | Grapevine |     |      | Almond |     |      |        |     |      |
| 'Figure 2A' | 92    | n/a |      | 'Figure 2B' | 24  | n/a | 'Figure 6' | 80  | n/a | 'Figure 9' | 336 | n/a | n/a |
| N         | -0.84 | 0.08| <0.01| -0.49 | 0.10| <0.01| -0.68 | 0.14| <0.01| -1.37 | 0.11| <0.01 |
| $\Theta_1$ (MPa) | -1.31 | 0.16| <0.01| -2.22 | 0.26| <0.01| -1.33 | 0.44| <0.01| -1.94 | 0.09| <0.01 |
| $\beta_1$ | 1.33  | 0.23| <0.01| 3.64  | 0.57| <0.01| 1.23  | 0.63| 0.05 | 0.98  | 0.19| <0.01 |
| $\beta_2$ | 0.09  | 0.20| <0.01| 0.49  | 0.63| 0.05 | -0.03 | 1.02| 0.17 | 0.23  | 0.20| <0.01 |
| $\beta_3$ | 0.73  | 0.07| <0.01| 1.80  | 0.95| <0.01| 1.39  | 0.86| 0.11 | 1.05  | 0.08| <0.01 |
| $\alpha$ (MPa) | -0.62 | 0.08| <0.01| 0.32  | 0.28| <0.01| 0.60  | 0.24| <0.01| 0.14  | 0.03| <0.01 |
| $R^2$     | 0.91  | n/a |      | 0.90  | n/a |      | 0.62  | n/a |      | 0.95  | n/a |      |
**Figure legends**

**Figure 1:** Relationship between plant water potential (Ψ) at predawn and midday for walnut trees (variety ‘Cisco’). Trees were subjected to a slow drydown (in A and B; controlled irrigation over weeks) or fast drydown (in C and D; i.e. no irrigation over days). Data are summarized for trees grafted on rootstock RX1, VX211 and Vlach. (A, C) The dashed line is a smoothed line (smoothing factor of sm=60) that best followed the pattern of data points. The solid line is a linear regression fitted across data points (in A, \( R^2 = 0.87 \), \( m = 0.75 \), \( P < 0.0001 \); in B, \( R^2 = 0.85 \), \( m = 0.89 \), \( P < 0.0001 \)). (C) During the fast drydown, same symbols indicate data collected for the same tree. (B, D) Relationship of predawn Ψ and slope values derived for the smoothed line in A and C. Parameters a and c are the maximum and b the minimum slope values, and \( \gamma_1 \) and \( \gamma_2 \) are the calculated transition points.

**Figure 2:** Piecewise linear regression model describing the triphasic relationship between predawn and midday Ψ for walnut trees. Model shown as bold solid line. Data are shown for trees (variety ‘Cisco’ grafted on rootstock RX1, VX211 and Vlach) subjected to a slow drydown (in A, corresponding to Figures 1A and 1B) or fast drydown (in B, corresponding to Figures 1C and 1D). (B) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II (\( \Theta_1 \)) and phase-II and -III (\( \Theta_2 \)) and corresponding standard errors are indicated in gray color. Model output parameters are summarized in Table 1.

**Figure 3:** Relationship between predawn Ψ and leaf gas exchange (stomatal conductance or \( \text{CO}_2 \) assimilation rate) for walnut trees. Trees (variety ‘ Cisco’ grafted on rootstock RX1, VX211 and Vlach) were subjected to a slow drydown (in A and B) or fast drydown (in C and D). (C, D) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve (corresponding to Figure 2). Vertically solid lines are the boundaries between phase-I and -II (\( \Theta_1 \)) and phase-II and -III (\( \Theta_2 \)) and corresponding standard errors are indicated in gray color. (A, B) The dashed line is a smoothed line (smoothing factor of sm=60) that best described the pattern of data points. (C, D) Due to the limit amount of data points collected during the fast drydown for phase-II and -III a fitted line is not included.

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Figure 4: Relationship between predawn $\Psi$ and leaf cell pressure (hydrostatic turgor or sap osmotic pressure) for walnut trees. Trees (variety ‘Cisco’ grafted on rootstock RX1, VX211 and Vlach) were subjected to a slow drydown (in A and B) or fast drydown (in C and D). (C, D) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve (corresponding to Figure 2). Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. (A, B) The dashed line is a smoothed line (smoothing factors of $sm=60$ in A and $sm=75$ in B) that best described the pattern of data points. The arrow in (A) indicates the average leaf turgor loss point ($\Psi_{TLP}$) of -1.4 ± 0.14 MPa as measured from pressure-volume curves (see Supplemental Table S1). (C, D) Due to the limited amount of data points collected during the fast drydown for phase-II and -III a fitted line is not included.

Figure 5: Relationship between predawn and midday $\Psi$ for grapevine (variety ‘Chardonnay’). Data are summarized for variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420. (A) The dashed line is a smoothed line (smoothing factor of $sm=60$) that best followed the pattern of data points. The solid line is a linear regression fitted across data points ($R^2 = 0.59, m = 0.79, P < 0.0001$). (B) Relationship of predawn $\Psi$ and slope values derived for the smoothed line in A. Parameters $a$ and $c$ are the maximum and $b$ the minimum slope values, and $\gamma_1$ and $\gamma_2$ are the calculated transition points.

Figure 6: Piecewise linear regression model describing the triphasic relationship between predawn and midday $\Psi$ for grapevine (variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420) corresponding to Figure 5. Model shown as bold solid line. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. Model output parameters are summarized in Table 1.

Figure 7: Relationship between predawn $\Psi$ and leaf gas exchange (stomatal conductance or $CO_2$ assimilation rate) for grapevine (variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420) corresponding to Figures 5 and 6. The dashed line is a smoothed line (smoothing factor of $sm=60$) that best described the pattern of data points. Since leaf gas exchange reached a minimum at predawn $\Psi$ of around -0.7 MPa, additional data were not collected past this point. Roman numerals I to III designate the three phases of the
Figure 8: Relationship between predawn and midday \( \Psi \) for almond (variety ‘Nonpareil’). Data were obtained for trees grafted onto rootstock Nemaguard (A) The dashed line is a smoothed line (smoothing factor of \( \text{sm}=60 \)) that best followed the pattern of data points. The solid line is a linear regression fitted across data points \( R^2 = 0.94 \), \( m = 0.79 \), \( P < 0.0001 \). (B) Relationship of predawn \( \Psi \) and slope values derived for the smoothed line in A. Parameters \( a \) and \( c \) are the maximum and \( b \) the minimum slope values, and \( \gamma_1 \) and \( \gamma_2 \) are the calculated transition points.

Figure 9: Piecewise linear regression model describing the triphasic relationship between predawn and midday \( \Psi \) for almond (variety ‘Nonpareil’ grafted on rootstock Nemaguard) corresponding to Figure 8. Model shown as bold solid line. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II \( (\Theta_1) \) and phase-II and -III \( (\Theta_2) \) and corresponding standard errors are indicated in gray color. Model output parameters are summarized in Table 1.

Figure 10: Scheme illustrating the stepwise procedure for measurement of plant water potential \( (\Psi) \). Bold arrows indicate the sequence of steps that is recommended for generation of the WP curve. The ‘equilibration’ step \( (>30 \text{ min}) \) ensures a final measurement of \( \Psi \) that best reflects the magnitude of negative xylem pressure. Dashed arrows indicate the sequence of alternative steps that may be followed to speed up the time between ‘covering’ and ‘excision’ \( (<10 \text{ sec}) \) and/or to allow for transport of leaf samples and extend the time period between ‘excision’ and ‘measurement’ to several hours.
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Figure 1: Relationship between plant water potential ($\Psi$) at predawn and midday for walnut trees (variety 'Cisco') subjected to a slow drydown (in A and B; controlled irrigation over weeks) or fast drydown (in C and D; i.e. no irrigation over days). Data are summarized for trees grafted on rootstock RX1, VX211 and Vlach. (A, C) The dashed line is a smoothed line (smoothing factor of sm=60) that best followed the pattern of data points. The solid line is a linear regression fitted across data points (in A, $R^2 = 0.87$ $m = 0.75$, $P < 0.0001$; in B, $R^2 = 0.85$, $m = 0.89$, $P < 0.0001$). (C) During the fast drydown, same symbols indicate data collected for the same tree. (B, D) Relationship of predawn $\Psi$ and slope values derived for the smoothed line in A and C. Parameters $a$ and $c$ are the maximum and $b$ the minimum slope values, and $\gamma_1$ and $\gamma_2$ are the calculated transition points.
Figure 2: Piecewise linear regression model (indicated as bold solid line) describing the triphasic relationship between predawn and midday Ψ for walnut trees. Data are shown for trees (variety ‘Cisco’ grafted on rootstock RX1, VX211 and Vlach) subjected to a slow drydown (in A, corresponding to Figures 1A and 1B) or fast drydown (in B, corresponding to Figures 1C and 1D). (B) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II (Θ₁) and phase-II and -III (Θ₂) and corresponding standard errors are indicated in gray color.

Model output parameters are summarized in Table 1.
Figure 3: Relationship between predawn $\Psi$ and stomatal conductance or CO$_2$ assimilation rate for walnut trees (variety ‘Cisco’ grafted on rootstock RX1, VX211 and Vlach) subjected to a slow drydown (in A and B) or fast drydown (in C and D). (C, D) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve (corresponding to Figure 2). Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. (A, B) The dashed line is a smoothed line (smoothing factor of sm=60) that best described the pattern of data points. (C, D) Due to the limit amount of data points collected during the fast drydown for phase-II and -III a fitted line is not included.
Figure 4: Relationship between predawn $\Psi$ and leaf turgor or sap osmotic pressure for walnut trees (variety 'Cisco' grafted on rootstock RX1, VX211 and Vlach) subjected to a slow drydown (in A and B) or fast drydown (in C and D). (C, D) During the fast drydown, same symbols indicate data collected for the same tree. Roman numerals I to III designate the three phases of the water potential curve (corresponding to Figure 2). Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. (A, B) The dashed line is a smoothed line (smoothing factors of sm=60 in A and sm=75 in B) that best described the pattern of data points. The arrow in (A) indicates the average leaf $\Psi_{\text{TLP}}$ of $-1.4 \pm 0.14$ MPa as measured from pressure-volume curves (see Supplemental Table S1). (C, D) Due to the limit amount of data points collected during the fast drydown for phase-II and -III a fitted line is not included.
Figure 5: Relationship between predawn and midday $\Psi$ for grapevine (variety ‘Chardonnay’). Data are summarized for variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420. (A) The dashed line is a smoothed line (smoothing factor of sm=60) that best followed the pattern of data points. The solid line is a linear regression fitted across data points ($R^2 = 0.59 \; m = 0.79, \; P < 0.0001$). (B) Relationship of predawn $\Psi$ and slope values derived for the smoothed line. $a$, $b$, $c$, $\gamma_1$, and $\gamma_2$ are the maximum and minimum slope values, and $\gamma_1$ and $\gamma_2$ are the calculated transition points.
**Figure 6:** Piecewise linear regression model (indicated by bold solid line) describing the triphasic relationship between predawn and midday $\Psi$ for grapevine (variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420) corresponding to Figure 5. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. Model output parameters are summarized in Table 1.
Figure 7: Relationship between predawn $\Psi$ and stomatal conductance or $CO_2$ assimilation rate for grapevine (variety ‘Chardonnay’ grafted on rootstock 110R, 1103, 140RU, 5C, RG, Ramsey, 101-14 and 420) corresponding to Figures 5 and 6. The dashed line is a smoothed line (smoothing factor of sm=60) that best described the pattern of data points. Since leaf gas exchange reached a minimum at predawn $\Psi$ of around -0.7 MPa, additional data were not collected past this point. Roman numerals I to III designate the three phases of the water potential curve (corresponding to Figure 6). Vertically solid lines are the modelled boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color.
Figure 8: Relationship between predawn and midday $\Psi$ for almond (variety ‘Nonpareil’). Data were obtained for trees grafted onto rootstock Nemaguard (A) The dashed line is a smoothed line (smoothing factor of $sm=60$) that best followed the pattern of data points. The solid line is a linear regression fitted across data points ($R^2 = 0.94, m = 0.79, P < 0.0001$). (B) Relationship of predawn $\Psi$ and slope values derived for the smoothed line in A. Parameters $a$ and $c$ are the maximum and $b$ the minimum slope values, and $\gamma_1$ and $\gamma_2$ are the calculated transition points.
Figure 9: Piecewise linear regression model (indicated by bold solid line) describing the triphasic relationship between predawn and midday $\Psi$ for almond (variety ‘Nonpareil’ grafted on rootstock Nemaguard) corresponding to Figure 8. Roman numerals I to III designate the three phases of the water potential curve. Vertically solid lines are the boundaries between phase-I and -II ($\Theta_1$) and phase-II and -III ($\Theta_2$) and corresponding standard errors are indicated in gray color. Model output parameters are summarized in Table 1.
Figure 10: Scheme illustrating the stepwise procedure for measurement of plant water potential (Ψ). Bold arrows indicate the sequence of steps that is recommended for generation of the WP-curve. The ‘equilibration’ step (>30 min) ensures a final measurement of Ψ that best reflects the magnitude of negative xylem pressure. Dashed arrows indicate the sequence of alternative steps that may be followed to speed up the time between ‘covering’ and ‘excision’ (releases) and/or to allow for transport of leaf samples and extend the time period between ‘excision’ and ‘measurement’ to several hours.
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