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Abscisic acid increase correlates with the soil water threshold of transpiration decline during drought

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Abstract

By regulating carbon uptake and water loss by plants, stomata are not only responsible for productivity but also survival during drought. The timing of the onset of stomatal closure is crucial for preventing excessive water loss during drought, but is poorly explained by plant hydraulics alone and what triggers stomatal closure remains disputed. We investigated whether the hormone abscisic acid (ABA) was this trigger in a highly embolism-resistant tree species *Umbellularia californica*. We tracked leaf ABA levels, determined the leaf water potential and gravimetric soil water content (gSWC) thresholds for stomatal closure and transpiration decline during a progressive drought. We found that *U. californica* plants have a peaking-type ABA dynamic, where ABA levels rise early in drought and then decline under prolonged drought conditions. The early increase in ABA levels correlated with the closing of stomata and reduced transpiration. Furthermore, we found that transpiration declined before any large decreases in predawn plant water status and could best be explained by transient drops in midday water potentials triggering increased ABA levels. Our results indicate that ABA-mediated stomatal regulation may be an integral mechanism for reducing transpiration during drought before major drops in bulk soil and plant water status.

KEYWORDS

ABA, dendrometer, soil moisture, stomata

1 | INTRODUCTION

Stomatal pores are the only means by which vascular plants can sustain high rates of gas exchange on land because they close to prevent excessive water loss, catastrophic embolism spread and irrecoverable tissue damage during drought (Brodribb & Holbrook, 2003; Brodribb et al., 2021). While stomatal closure is essential for survival in dry environments, there is considerable ecological variation in stomatal sensitivity to drought between species (Brodribb & McAdam, 2013; Hochberg et al., 2018; Jin et al., 2023; Martínez-Vilalta & Garcia-Forner, 2017; Martin-StPaul et al., 2017; McDowell et al., 2008). With the expected changes in drought intensity, frequency and distribution, accurately predicting the timing and dynamics of stomatal closure during drought is critical for understanding future plant responses to the environment (Cochard et al., 2021). Most plants show a decrease in stomatal conductance as drought progresses; often modelled as an exponential decline with decreasing plant water potential during drought (Dewar, 2002; Martínez-Vilalta & Garcia-Forner, 2017; Tardieu et al., 1998). This decrease in stomatal conductance can be observed occurring over a short range of water potential decrease (Brodribb & Holbrook, 2004), yet the water potential at which stomata close is highly variable and is argued to be one of the most important determinants of plant survival during drought (Bartlett et al., 2012; Blackman et al., 2023). Despite the importance of this response, the mechanism driving stomatal closure during drought and thus governing variation in stomatal regulation within and across species (Hochberg et al., 2018) remains ambiguous, ranging from passive turgor loss of guard cells (Mott & Peak, 2013; Oren et al., 1999; Tombesi et al., 2015) to decreases in guard cell turgor triggered by metabolic signals, like the hormone abscisic acid (ABA) (Dodd, 2003; Sussmilch et al., 2017a).

During a drought, declines in leaf water status are considered the simplest mechanistic cause of decreases in guard cell turgor and subsequent stomatal closure. This simple, passive mechanism of stomatal regulation is readily observed in species of lycophyte and fern, in which stomatal responses to declines in leaf water status are highly predictable (Brodribb & McAdam, 2011; Cardoso et al., 2019; Gong et al., 2021; Martins et al., 2016). Studies exploring hydraulic drivers of stomatal closure in seed plants also find stomata close as leaf water status declines (Carminati & Javaux, 2020; Cochard & Delzon, 2013; Hochberg et al., 2017). Yet, most mechanistic studies and models of stomatal response to water status in seed plants show that a simple, hydropassive coupling of guard cell turgor with leaf water status is not enough to cause stomatal closure, and only a biochemically-active mechanism can cause the needed decreases in guard cell turgor (Buckley, 2005; Delwiche & Cooke, 1976; Franks & Farquhar, 2007). This is supported by observations that mutants defective in ABA signalling or biosynthesis have highly dysfunctional stomatal responses to water status, with plants carrying mutations in multiple genes in these pathways having stomata that are profoundly insensitive to changes in leaf water status (Brodribb et al., 2021; Holbrook et al., 2002; Sussmilch et al., 2017b). Other evidence for an active regulation of stomatal response to drought in seed plants comes from the nature of stomatal dynamics. During drought, in seed plants there is a period when stomata regulate transpiration before minimum or complete stomatal closure is achieved, a phase of drought that has been termed ‘early stomatal closure’, a phenomenon well-studied in crop species (Gambetta et al., 2020; Koehler et al., 2023; Sinclair et al., 2015). Purely passive-hydraulic mechanistic drivers of stomatal closure cannot easily explain early stomatal closure without evoking profound, yet rapidly reversible, losses of hydraulic conductivity along some path of the soil–plant–atmosphere continuum (SPAC) (Rodriguez-Dominguez & Brodribb, 2020; Scoffoni et al., 2023; Sperry, 2000). These range from a loss of outside xylem conductivity in the leaf (Scoffoni et al., 2023; Yaaran et al., 2023; Zait et al., 2017); embolism in the xylem that refills (Sperry, 2000); hydraulic isolation of the root from the soil (Cuneo et al., 2016; Rodriguez-Dominguez & Brodribb, 2020); or loss of soil hydraulic conductivity (Carminati & Javaux, 2020; Carminati et al., 2020; Koehler et al., 2022). While there are a lot of potential breakpoints in the SPAC that could drive stomatal closure many remain a matter of debate; for instance, xylem refilling under tension does not occur (Charrier et al., 2016) so is no longer an adequate explanation for early stomatal closure.

In the absence of any major declines in plant hydraulic conductivity, our understanding of turgor pressures in guard cells suggest that a metabolic trigger for stomatal closure during drought is essential. ABA is widely accepted as a primary metabolic trigger of stomatal closure in seed plants (Buckley, 2019; Davies & Zhang, 1991; Kriedemann et al., 1972; McAdam & Brodribb, 2015; Mittelheuser & Steveninck, 1969), but whether ABA plays a role in the initiation of stomatal closure is widely debated (Tombesi et al., 2015). There is very little incorporation of a modern understanding of ABA synthesis and signalling into the regulation of transpiration. Roots and xylem were once considered to be the potential sites of ABA production during drought (Jiang & Hartung, 2008), but modern evidence indicates that ABA biosynthesis primarily occurs in leaves (Audran et al., 1998; Christmann et al., 2005; Holbrook et al., 2002; Ikegami et al., 2009; Manzi et al., 2015). A few models for stomatal function have included ABA response either as a fitted parameter (Buckley & Mott, 2013; Rodriguez-Dominguez et al., 2016) or as a xylem delivered function of root water potential (Tardieu et al., 2015), and as such are not yet applicable for predicting leaf endogenous ABA levels during drought. In addition, thresholds of soil and plant water potentials are predicted to depend on stomatal closure (Carminati et al., 2020) rather than act as predictors of stomatal closure, making it important to resolve the role of ABA dynamics as a regulator of transpiration during drought.

A key piece of information lacking from studies which document stomatal sensitivity to water limitation is a link to ABA dynamics (Tombesi et al., 2015). Here we test whether the initiation of stomatal closure during drought correlates with increases in leaf ABA levels in the extremely drought-tolerant species *Umbellularia californica*. We choose this species because it has highly embolism-resistant xylem, with embolism occurring at very negative water potentials (<−6 MPa), and it has recently been hypothesized that increased ABA levels early during a drought closes stomata, with a peaking-type dynamic after which ABA levels decline even as drought progresses (Mercado-Reyes et al., 2023). In this study we used this model species to test the hypothesis that increased ABA levels correlate with early stomatal closure during drought. We describe the relationship between declines in canopy conductance and water potentials, and increases in endogenous ABA levels during drought.

2 | MATERIALS AND METHODS

Plants were grown from seed in a 1:2:1 ratio of Indiana Miami topsoil (a fine-loamy, mixed, active, mesic Oxyaquic Hapludalf), ground pine bark and sand. Plants were 6 years of age and maintained in 8-L pots in greenhouse conditions set at 22/19°C day/night temperature under natural light, mean vapour pressure deficit (VPD) was 1.13 kPa ranging from 0.25 to 1.61 kPa (Supporting Information S1: Figure S1). The experiment was started on the autumnal equinox in 2022. Plants were watered daily and fertilized weekly with liquid nutrients (1 g per

4 L; 20:20:20 N:P:K; Miracle-Gro Water-soluble All Purpose Plant Food, Scotts Company LLC).

2.1 | Drought treatment

Plants were first watered beyond pot capacity in the evening before the experiment. In the morning, when soil was saturated and had stopped leaking water, total pot mass was recorded. Pots were double bagged to prevent any water loss from the soil. Daily predawn pot weights were measured. To impose an even and progressive drought on four plants, pots were re-watered so that daily available pot water did not decrease by more than 200 g from the previous day. To account for day-to-day environmental effects, including variation in light intensity, two additional plants were kept under well-watered conditions by rewatering pots to stay within 200 g of mass at maximum soil water holding capacity. Daily transpiration for these plants for the duration of the drought is shown in Supporting Information S1: Figure S1.

2.2 | Measurements

Water loss measured from daily pot weights was considered to be the 24-h transpiration rate for each plant. Relative transpiration was found to account for both day-to-day and plant-to-plant variation. Daily transpiration ratio (DTR) was first found by normalizing transpiration by the average well-watered transpiration of the day. The daily relative transpiration was found by normalizing the DTR by the average pot specific DTR over the first 3 days when transpiration levels were equivalent to well-watered plants. Leaves were counted for each plant twice, first before and second, after the experiment concluded. The number of leaves destructively sampled during the experiment was accounted for in the final leaf count. All increases in leaf number were considered to occur before drought-induced declines in transpiration began; since it has been shown that during drought, phyllochron index stops increasing before transpiration declines begin (Manandhar et al., 2017). The average rate of leaf addition per day for the duration over which transpiration remained maximum was projected as a linear increase in leaf number with days until the day transpiration decline began. After this day leaf count was considered to remain constant. At the end of the experiment, a representative sample of 180 leaves was used to find the average leaf area. The average leaf area and estimated leaf count for each plant was used to calculate a dynamic leaf area for the duration of the experiment (Supporting Information S2: Table S1). Canopy conductance was calculated from daily transpiration, leaf area and average daily VPD, calculated from air temperature and humidity data logged every minute for the duration of the experiment, from a sensor protected from direct sunlight, suspended at canopy height, using the following equation:

$$g_c = E \times \frac{P_{atm}}{\mathrm{VPD}},$$

where E is the water loss per unit leaf area per unit time and P_{atm} is atmospheric pressure at 170 m above sea level.

gSWC values were measured at two timepoints for each pot. First, the final pot weight (PW_f), at the end of the drought, and the corresponding gSWC ($gSWC_f$) were measured. To measure gSWC, a subsample of approximately 30 g soil was destructively taken from each pot making sure to not include any roots in the sampling. The subsamples of soil were weighted, dried for 3 days at 105°C and reweighed to measure dry soil weight. The dry soil weight was subtracted from the initial wet soil weight to find the mass of water for each soil sample. The ratio of the mass of water to the mass of dry soil was calculated as gSWC. The pots were rewatered to pot capacity, weighed (PW_{pc}), and another subsample of soil was taken from to get gSWC at PW_{pc} ($gSWC_{pc}$). The plant available soil water (ASW) at PW_f was set as zero. gSWC during the drought was calculated using the following equation:

$$\mathrm{gSWC} = \mathrm{ASW} \times \frac{\mathrm{gSWC}_{pc} - \mathrm{gSWC}_f}{\mathrm{PW}_{pc} - \mathrm{PW}_f} + \mathrm{gSWC}.$$

Since the total weight of water in the canopy, estimated from pressure–volume curves, never exceeded 1.5% of the total pot weight, we attributed all changes in pot weight to water loss from the soil.

Leaf predawn water potentials (Ψ_{pd}) were measured every 2 days, using the Scholander pressure chamber. Leaves were excised and immediately wrapped in a damp paper towel and sealed in a small ziplock bag for equilibration (at least 10 min). To reduce large reductions in canopy area midday water potentials (Ψ_{md}) were measured using the Scholander pressure chamber every 3 days initially, increasing to every other day when transpiration started decreasing.

In addition to point measurements of leaf water potentials (Ψ_{leaf}), optical dendrometers were installed below the leaf apex (Supporting Information S1: Figure S2) in each plant to determine dynamic changes in Ψ_{leaf} according to the method of Bourbia et al. (2021). Since *U. californica* plants have very short petioles, the optical dendrometers were instead set to image the leaf lamina, and rather than width we used surface area. Images were taken every 5 min. Optical dendrometers were installed while relative transpiration still matched well-watered plants. Plant specific relationships between leaf area and measured Ψ_{leaf}, using the Scholander pressure chamber, were established and used to determine Ψ_{leaf} dynamics during the drought (Supporting Information S1: Figure S3).

2.2.1 | Quantification of ABA levels during drought

Approximately 4 cm^2 of leaf area was taken from leaves after pressure chamber determination of Ψ_{leaf} and covered in 80% methanol in water ($v\,v^{-1}$) with added butylated hydroxytoluene (250 $mg\,L^{-1}$), roughly chopped and stored at −20°C. To prepare samples for ABA quantification, tissue was homogenized and 15 ng of labelled [$^2H_6$]-ABA was added to each sample as an internal standard. ABA was extracted from homogenized tissue overnight at 4°C after which an aliquot of the supernatant was dehydrated at 40°C, the ABA resuspended in 200 µL of 2% acetic acid in water ($v\ v^{-1}$) and ABA levels quantified using liquid chromatography tandem mass spectrometry (Agilent 6460, QQQ LCMS).

2.2.2 | Determination of leaf turgor loss point

To determine Ψ_{leaf} at turgor loss point (Ψ_{tlp}) pressure volume curves were measured in four leaves taken from four plants according to the method of Tyree and Hammel (1972).

2.3 | Analysis

2.3.1 | Leaf water potential

The initial dendrometer determined leaf area of hydrated plants at predawn was considered the maximum for each plant. All subsequent images of leaf area were expressed relative to this maximum, and were subsequently considered relative dendrometer area. A quadratic plateau relationship (package: rcompanion 2.4.34, R version 4.3.1) between Ψ_{leaf} and the relative leaf area at the time of measurement was fitted to predict Ψ_{leaf} over the course of the drought. The variation introduced by using leaf area instead of petiole width to estimate Ψ_{leaf} required a plant specific relationship between relative dendrometer area and measured Ψ_{leaf} (Supporting Information S1: Figure S3).

2.3.2 | Data analysis

When relationships between drought level and observed variables were nonlinear, data were visualized with an exponential regression for canopy conductance against Ψ_{md} and loess smoothing (locally linear smoothing) for the trends in ABA levels relative to gSWC during drought using the ggplot2 package (version 3.4.0) in R 4.12.1. The threshold gSWC that initiates declines in relative transpiration and canopy conductance was found using a segmented linear regression with the segmented package (version 1.6-1) in R. The significance of all thresholds was confirmed using a Davies test in R (Supporting Information S1: Table 2).

3 | RESULTS

U. californica plants had an initial, well-watered mean canopy conductance of 124 ± 2.5 $mmol\ m^{-2}\ s^{-1}$ ($n = 6$, ±SE) that decreased in the water-stressed plants to 3.59 ± 0.79 $mmol\ m^{-2}\ s^{-1}$ ($n = 4$, ±SE) over 36 days (Figure 1). An exponential relationship best described the decrease in canopy conductance with decreasing Ψ_{md} ($g_s = 150.5 \times e^{\psi_{md} \times 0.84}$), $R^2 = 0.68$, $p < 0.0001$) (Figure 1). Ψ_{pd} under well-watered conditions was −0.40 ± 0.05 MPa ($n = 6$, ±SE) and plants under drought stress decreased to a mean minimum of −3.75 ± 0.17 MPa ($n = 4$, ±SE). Mean Ψ_{md} decreased from −1.36 ± 0.06 MPa ($n = 6$, ±SE) in well-watered plants to a minimum of −4.76 ± 0.23 MPa ($n = 4$, ±SE) in the drought-stressed plants. Mean leaf turgor loss point was −1.84 ± 0.24 MPa ($n = 4$, ±SE).

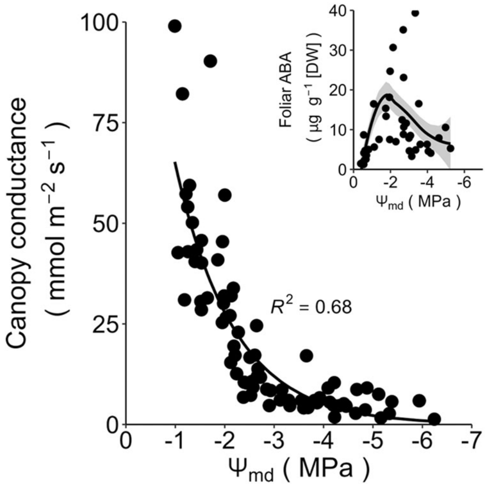

FIGURE 1 The exponential relationship between canopy conductance and dendrometer measured midday leaf water potential (Ψ_{md}) during soil drying in *Umbellularia californica*. Canopy conductance is the average vapour conductance estimated from the total daily water loss and average vapour pressure deficit for a given day. The inset shows the leaf levels of abscisic acid (ABA) as a function of Ψ_{md} during the drought. A loess (locally linear smoothing) regression the trend of ABA levels as Ψ_{md} decreases, with the shaded region showing the standard error of the ABA trend.

Average daily transpiration declined from 0.81 ± 0.1 $mmol\ m^{-2}\ s^{-1}$ when plants were still well-watered to 0.025 ± 0.01 $mmol\ m^{-2}\ s^{-1}$, at the end of the drought in the stress treatment. As gSWC declined, canopy conductance varied around well-watered levels over a wide range of gSWC until 0.32 gSWC (95% CI: 0.24–0.33), after which water limitation caused decreases in canopy conductance. A similar trend was observed when transpiration was normalized to account for day-to-day environmental variation and plant-to-plant variation. Relative transpiration remained constant and similar to well-watered conditions during the early part of the drought and only decreased significantly after gSWC declined below 0.32 (95% CI: 0.30–0.34) (Figure 2a). When the soil dried beyond 0.32 gSWC, transpiration decreased from 100% to 10% within a period of 4.80 ± 0.85 days ($n = 4$, ±SE).

Mean leaf ABA levels increased at the same threshold of 0.32 gSWC (Figure 2a). A peaking-type dynamic of ABA was also clear when leaf ABA levels were expressed relative to gSWC (Figure 2a). The highest mean ABA levels of 32.1 ± 6.2 $\mu g\ g^{-1}$ dw ($n = 4$, ±SE) were seen when relative transpiration was reduced to 27 ± 12% of initial transpiration at a gSWC of 0.17 ± 0.03. ABA levels decreased after this peak and when transpiration rate approached 11 ± 1.7% ABA levels declined to 4.34 ± 0.46 $\mu g\ g^{-1}$ dw. Mean ABA levels under well-watered conditions (3.09 ± 0.79 $\mu g\ g^{-1}$ dw) were the same as

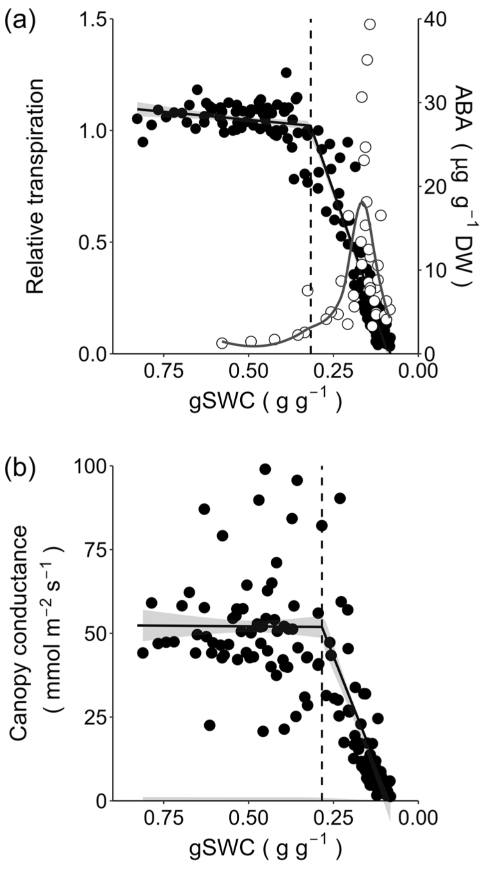

FIGURE 2 (a) The total daily transpiration relative to well-watered conditions and leaf abscisic acid (ABA) levels as gravimetric soil water content (gSWC) decreases during drought in *Umbellularia californica*. Each filled circle is the relative transpiration for one plant over 24 h and each empty circle is the ABA level of one leaf at the corresponding gSWC. (b) Canopy conductance as gSWC decreases, where each filled circle is the conductance of one plant determined from daily transpiration and plant leaf area. A two segmented linear regression and standard error (shaded region) is fit to show the trend of relative transpiration and canopy conductance as gSWC decreases. The break point for each relationship is indicated by the vertical dashed line. A loess (locally linear smoothing) regression depicts the trend of ABA levels as gSWC declines.

ABA levels at the end of the drought ($p = 0.17$, paired t-test for difference in mean).

Initially, Ψ_{pd} and Ψ_{md} declined gradually as soil dried. Only after the soil dried past 0.20 (95% CI: 0.18–0.21) gSWC did Ψ_{pd} decrease steeply, while Ψ_{md} decreased steeply after a gSWC of 0.18 (95% CI: 0.16–0.19) (Figure 3). The gSWC threshold of decline for Ψ_{pd} and Ψ_{md} were not significantly different based on the 95% confidence intervals. This steep decline in water potential occurred 4.75 ± 0.85 ($n = 4$, SE) days after the declines in transpiration began. Leaf area measured by the dendrometer displayed a strong relationship with Ψ_{leaf} measured with the Scholander pressure chamber on a plant-by-plant basis (Supporting Information S1: Figure S3). Decreases in Ψ_{leaf} during drought were well-reflected in both the measured and dendrometer predicted Ψ_{leaf}.

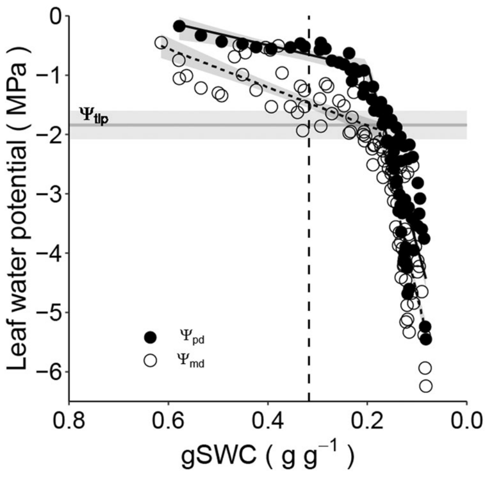

FIGURE 3 Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential as gravimetric soil water (gSWC) content decreases during drought in *Umbellularia californica*. The filled circles are the Ψ_{pd} and empty circles are the Ψ_{md}. Continuous data of dendrometer determined leaf water potential (Supporting Information S1: Figure S4) were used to determine Ψ_{pd} and Ψ_{md}. The black solid and dotted lines show two segmented linear regressions with standard error (shaded region). Mean water potential at turgor loss point is shown (grey horizontal line with shaded area depicting standard error). The vertical dashed line is the gSWC threshold at which transpiration begins to decline (from Figure 2a).

4 | DISCUSSION

Many studies have shown in angiosperms that canopy conductance declines exponentially with declining Ψ_{leaf} during drought (Dewar, 2002; Martínez-Vilalta & Garcia-Forner, 2017; Tardieu et al., 1998). We observed a similar response of stomata to Ψ_{leaf} during drought in *U. californica* (Figure 1), but also a distinct soil water threshold of stomatal regulation during drought. We found that transpiration, and canopy conductance, remained constant until gSWC declined to 0.32 (Figure 2 and Supporting Information S1: Figure S4). It should be noted that gSWC is not volumetric soil water content, and can only be used to compare the relative timing of events in this experiment. The gSWC threshold at which stomata closed during drought preceded the threshold gSWC at which Ψ_{pd} declined during drought, which occurred at around 0.20 gSWC. We find that this reduction in maximum transpiration before rapid declines in Ψ_{pd} conserves plant and soil water status, prolonging survival during drought (Carminati et al., 2020). Stomata closed at a threshold gSWC that preceded any declines in the hydraulic capacity of the xylem; the water potential at 50% loss of conductivity for this species has been reported to be −7.5 ± 0.47 MPa (Mercado-Reyes et al., 2023).

Soil water content thresholds for stomatal closure during drought have been observed across a number of herbaceous crop species (Koehler et al., 2022, 2023; Manandhar et al., 2017; Shekoofa et al., 2013; Sinclair, 2005; Sinclair & Ludlow, 1986). Inter- and intraspecific variation in the soil water threshold of stomatal closure has long been challenging for ecophysiologists to explain. A hydraulic failure explanation has been used to explain declines in transpiration during drought, although the cause of the loss of conductivity in the SPAC leading to a breakpoint remains debated (Bourbia et al., 2021; Carminati & Javaux, 2020; Carminati et al., 2020; Rodriguez-Dominguez & Brodribb, 2020; Wankmüller & Carminati, 2022). It has been argued that stomatal closure during drought is caused by a failure of the root–soil interface (Rodriguez-Dominguez & Brodribb, 2020), yet our observed gSWC threshold for Ψ_{pd} decline suggests that stomatal closure begins earlier and progresses more gradually during drought than could be adequately explained by an unrecoverable decline in the hydraulic conductance of the root–soil interface alone. This includes any unrecoverable hydraulic declines in the rhizosphere as well. Though Ψ_{pd} may not be an exact approximation of soil water potential, it remains the most commonly used approximate, especially to integrate the heterogeneity of water in potted soil (Couvreur et al., 2012; Koehler et al., 2023). An alternative explanation that does not require unrecoverable declines in belowground hydraulic conductance is a hormonal regulation of stomata caused by a transient decline in hydraulic conductance somewhere in the SPAC.

ABA activates anion channels in guard cell membranes to decrease guard cell turgor, independently of leaf turgor (Ache et al., 2010; Bauer et al., 2013). We show that foliage ABA levels in *U. californica* start to increase as gSWC declines to the threshold gSWC at which transpiration declined, this differs from observations in grape in which ABA levels only increased significantly after complete stomatal closure (Tombesi et al., 2015). In *U. califonica* ABA levels remained low when transpiration was not regulated. Once triggered, ABA levels continued to increase until transpiration was reduced to 27% of well-water conditions. After this point stomata transitioned to being closed passively as ABA levels declined in this model peaking-type species (McAdam et al., 2023). This differs from species in which ABA levels continue to increase during drought, for which ABA actively closes stomata for the duration of a drought (Brodribb & McAdam, 2013). We believe that the regulation of transpiration by ABA delays declines in bulk gSWC and Ψ_{leaf}. To date, very few models for transpiration incorporate ABA dynamics, some assume ABA is derived from the roots (Tardieu et al., 2015), which has poor experimental support; while others assume that ABA levels reduce guard cell turgor, but do not predict ABA levels (Buckley & Mott, 2013). Most recently, Wankmüller and Carminati (2022) devised a model that predicts ABA levels regulate stomata and are a function of Ψ_{leaf} and assimilation. This model is more accurate than previous models that incorporate ABA in regulating stomatal responses to water status, but is limited to requiring highly accurate determination of minimum midday Ψ_{leaf}, and does not consider that ABA biosynthesis requires a drop in turgor pressure (McAdam & Brodribb, 2016; Pierce & Raschke, 1980, 1981). Continuous monitoring of Ψ_{leaf} during drought using optical dendrometers suggests that the time of minimum Ψ_{md} can occur over a very brief period of the day (sometimes even only 15 min, Figure 4). ABA biosynthesis in angiosperms can occur within 15 min of a decline in leaf turgor (Sussmilch et al., 2017b).

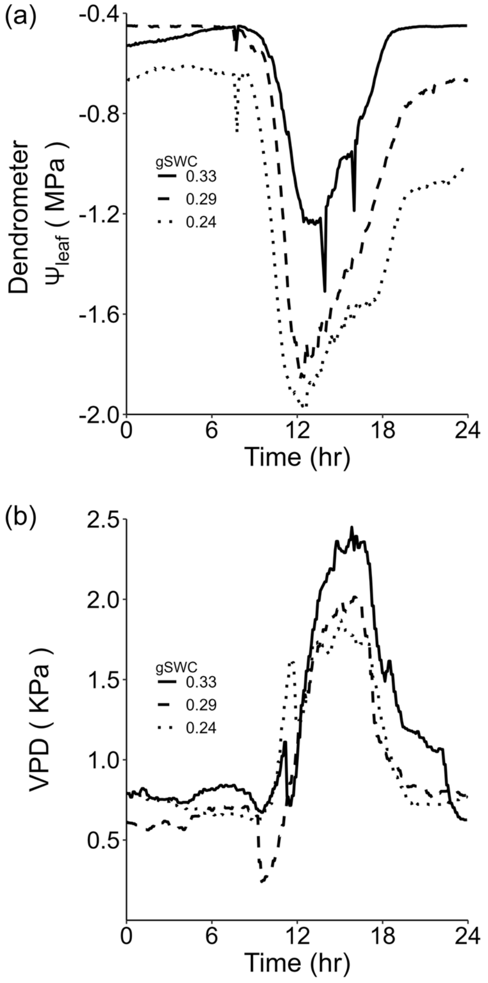

FIGURE 4 (a) The diurnal trends of dendrometer measured leaf water potential (Ψ_{leaf}) for the same representative plant of *Umbellularia californica* as gravimetric soil water content (gSWC) declines past the threshold of transpiration decline at 0.32 gSWC, three sequential days are shown. (b) Vapour pressure deficit (VPD) for each day is shown.

The trigger point of endogenous ABA production and early stomatal closure is linked to soil–plant water status during drought via leaf turgor. Since ABA production begins before severe incursions in bulk soil and plant water potentials, ABA production is likely occurring during transient drops in Ψ_{leaf} that might not be detected by single point measurements of Ψ_{leaf}. Mesophyll production of ABA caused by water loss is likely associated with decreases in turgor past a threshold (McAdam & Brodribb, 2016; Pierce & Raschke, 1980). While simple, it is not correct to assume that ABA production is triggered at turgor loss point; triggers of ABA production could be: a turgor differential, or at a threshold turgor point higher than turgor loss point, or even a loss of relative water content (Sack et al., 2018). Unfortunately, experiments to directly test these hypotheses are challenging, possibly involving pico-probes and the collection of very small amounts of cytoplasm to detect small changes in ABA levels (McAdam & Brodribb, 2016). In *U. californica* ABA levels increased when Ψ_{md} transiently declined below Ψ_{tlp} causing declines in transpiration during drought (Figures 2 and 3).

Increased ABA levels might also explain previously described differences in the rate of decline between Ψ_{md} and Ψ_{pd} (Knipfer et al., 2020) as gSWC declines (Figure 3). We found that Ψ_{md} declined more rapidly as gSWC declined than Ψ_{pd} which remained relatively constant early in the drought (Figure 3). This suggests that transient decreases in mesophyll water status and turgor could occur, at times when transpirational demand on leaf water exceeds the supply from the rhizosphere, triggering ABA biosynthesis. Differentials in soil water potential between the bulk soil and soil near the roots are thought to increase with transpiration (Carminati et al., 2020; Gardner, 1960). The critical water potential at which stomatal closure occurs is believed to be the water potential of the rhizosphere, this water potential is not reflected in bulk soil water status because of the transpiration driven differential within the soil (Carminati et al., 2020). Rhizosphere dehydration might lower conductivity with declines in Ψ_{md} occurring during drought even as Ψ_{pd} remains hydrated (Figures 3 and 4a). The decline in Ψ_{md} likely triggered ABA biosynthesis because it declines below Ψ_{tlp} for at least 20 min (Sussmilch et al., 2017b).

In conclusion, we find that there is a threshold gSWC at which stomata are regulated during drought and that the hormone ABA likely drives this. We also suggest that declines in conductivity through dry soil may trigger midday synthesis of ABA via Ψ_{leaf} which might explain observations of midday depression of stomatal conductance in the absence of declines in Ψ_{pd}. There is ongoing debate about whether complete failure of the root–soil interface occurs during drought (Carminati et al., 2020; Rodriguez-Dominguez & Brodribb, 2020); further work is required to resolve these questions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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