ORIGINAL RESEARCH

Physiologia Plantarum

Relationships between xylem embolism and tree functioning during drought, recovery, and recurring drought in Aleppo pine

Yael Wagner[1] | Mila Volkov[1] | Daniel Nadal-Sala[2,3] | Nadine Katrin Ruehr[2] | Uri Hochberg[4] | Tamir Klein[1]

[1]Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

[2]Institute of Meteorology and Climate Research (IMK-IFU), KIT-Campus Alpin, Karlsruhe Institute of Technology (KIT), Garmisch-Partenkirchen, Germany

[3]Centre de Recerca Ecològica i Aplicacions Forestals (CREAF), Campus de Bellaterra (UAB) Edifici C, Cerdanyola del Vallès, Spain

[4]Institute of Soil, Water and Environmental Sciences, Volcani Center, Agricultural Research Organization, Rishon LeZion, Israel

Correspondence
Tamir Klein, Department of Plant and Environmental Sciences, Weizmann Institute of Science, 234 Herzl Street, 76100 Rehovot, Israel.
Email: tamir.klein@weizmann.ac.il

Abstract

Recent findings suggest that trees can survive high levels of drought-induced xylem embolism. In many cases, the embolism is irreversible and, therefore, can potentially affect post-drought recovery and tree function under recurring droughts. We examined the development of embolism in potted Aleppo pines, a common species in hot, dry Mediterranean habitats. We asked (1) how post-drought recovery is affected by different levels of embolism and (2) what consequences this drought-induced damage has under a recurring drought scenario. Young trees were dehydrated to target water potential (Ψ_x) values of −3.5, −5.2 and −9.5 MPa (which corresponded to ~6%, ~41% and ~76% embolism), and recovery of the surviving trees was measured over an 8-months period (i.e., embolism, leaf gas-exchange, Ψ_x). An additional group of trees was exposed to Ψ_x of −6.0 MPa, either with or without preceding drought (Ψ_x of −5.2 MPa) to test the effect of hydraulic damage during repeated drought. Trees that reached −9.5 MPa died, but none from the other groups. Embolism levels in dying trees were on average 76% of conductive xylem and no tree was dying below 62% embolism. Stomatal recovery was negatively proportional to the level of hydraulic damage sustained during drought, for at least a month after drought relief. Trees that experienced drought for the second time took longer to reach fatal Ψ_x levels than first-time dehydrating trees. Decreased stomatal conductance following drought can be seen as "drought legacy," impeding recovery of tree functioning, but also as a safety mechanism during a consecutive drought.

1 | INTRODUCTION

Xylem embolism is a process in which air bubbles block the water-conducting elements of a plant. This happens when the tension in the functional xylem conduits becomes extreme, either due to a decrease in soil water availability or increased atmospheric demand (i.e., vapor pressure deficit; VPD). At a certain threshold, an air bubble is sucked from a non-functional conduit (at atmospheric pressure) into a functional conduit (under negative pressure), blocking the latter and impeding its hydraulic conductivity (i.e., "embolism"; Zimmermann, 1983), often in a non-reversible manner (Choat et al., 2018). Previous works have found 60%–90% loss of hydraulic conductivity (Adams et al., 2017; Barigah et al., 2013; Hammond et al., 2019) to be the "lethal dose" of embolism for different tree species, with anecdotal

occurrences of trees surviving as high as 96% embolism (Hammond et al., 2019). Specifically for Aleppo pine, García de la Serrana et al. (2015) observed >80% loss of hydraulic conductivity in declining, mature trees in semi-arid forests. It has been suggested that the key parameter for drought survival is the vitality of tissues essential for recovery, like the vascular cambium, parenchyma, and meristems (Körner, 2019; Mantova et al., 2021). Hammond et al. (2019) observed that as long as the water supply to the vascular cambium remained intact, Loblolly pine trees were able to recover from extreme levels of embolism. Furthermore, older vessels tend to have lower conductivity, so they contribute significantly less than newly formed vessels to xylem water transport (Bodo & Arain, 2021; Phillips et al., 1996). Thus, it is potentially the proximity of embolism to the cambium, rather than the sheer volume of embolism, that should be examined in the context of drought survival versus drought mortality.

Limited damage to the water-conducting system of the tree may not pose a large obstacle during routine drought since transpiration is minimized following stomatal closure and the demand for hydraulic conductivity is extremely low (Körner, 2019). However, assuming that embolized vessels are not routinely refilled (Cochard & Delzon, 2013), the loss of water-conducting tissues might limit the ability of the trees to resume carbon assimilation after drought has been relieved, until new xylem conduits are produced (Ruehr et al., 2019), especially if drought-induced defoliation has also occurred (Nadal-Sala et al., 2021). In fact, Rehschuh et al. (2020) suggested that while Scots pines are able to survive up to 50% embolism, their recovery is partial and proportional to the loss of hydraulic conductivity. This means that the tree's hydraulic conductivity might continue to limit post-drought leaf gas exchange recovery long after soil water availability has been restored.

If carbon assimilation rates or reserves do not match the carbon amount needed to rebuild new conductive tissue, trees may be unable to reach the pre-drought conditions, thus becoming more vulnerable to future drought episodes (Hammond, 2020; Kannenberg et al., 2020). And yet, there is potentially another aspect to drought-induced embolism. While decreased stomatal conductance (g_s) inhibits sugar accumulation, it also reduces water loss through transpiration. When considering ecosystems with recurring drought, like the Mediterranean, such stomatal downregulation might be beneficial to the next drought event.

In this study, we have experimentally imposed three levels of drought intensity on potted Aleppo pine trees (Pinus halepensis). We then measured the physiological parameters of the trees, including embolism level and distribution, as well as stomatal activity, at the peak of drought and through 8 months of rehydration, to determine the effect of the hydraulic damage sustained during drought on recovery. To understand the effect of repeated droughts, we have also implemented two consecutive drought periods for some of the trees. That is, one group of trees was dehydrated while the other was kept hydrated, and then all trees (from both groups) were dehydrated to fatal Ψ_x values to determine the effect of a first drought event on tree dehydration dynamics during a second drought. We hypothesized that (1) Aleppo pines, similarly to other pine species, would have a high tolerance to embolism, that is ~80%; (2) trees that have experienced hydraulic damage during drought would have impaired ability to recover physiological activity following drought release; and (3) trees that were previously exposed to drought would present a slower dehydration upon a second drought compared to trees have never experienced drought.

2 | MATERIALS AND METHODS

2.1 | Drought intensity effect

Thirty-one young *P. halepensis* trees (~2 years old) were obtained from the nursery of the Jewish National Fund (JNF, the Israeli forestry agency, Eshtaol, Israel). The trees were potted in 10 L pots of standard planting media (Even Ari Green) and grown under field conditions for ~6 months at the campus of the Weizmann institute, Rehovot, Israel. To examine the effect of drought intensity on recovery, the trees were dehydrated for different time-periods by withholding irrigation for 10 days ($n = 5$) or 25 days ($n = 16$). An additional group of trees ($n = 10$) was a well-irrigated control group, which was kept hydrated using drip irrigation (8 L hour^{-1}, Netafim Ltd.; the length of irrigation interval was adjusted instantaneously to maintain the pots at field capacity). These treatments resulted in average midday water potential (Ψ_x) values of -3.5 MPa (SE = 0.3 MPa) following 10 days drought, and either -5.2 MPa or -9.5 MPa (SE = 0.5 MPa and 0.6 MPa, respectively) in trees that were dehydrated for 25 days and either survived or died, respectively. These Ψ_x values corresponded to a loss of 6%, 41% and 76% of the xylem tissue to embolism. Ψ_x was measured at least once a week on at least one individual from each treatment group to monitor drought progress. Campaign days were conducted for each treatment group at the peak of drought, and then after 1 week, 2 weeks, 1 month, and 8 months of rehydration. During these, all trees were measured for Ψ_x, leaf gas exchange and embolism level. In addition, pressure–volume (PV) curves were constructed during peak drought campaigns for each of the groups. See Figure 1 for a schematic description of the experimental layout, and the following sections for detailed description of the measurements.

2.2 | Repeated drought effect

To test the effect of repeated drought on the trees, 10 individuals from the same batch as the first experiment were used in a consecutive experiment, where 5 trees were dehydrated for 20 days (on average—to P_{50} values), while the other 5 were kept irrigated. Then, all trees were irrigated for 2 weeks to allow for full Ψ_x recovery of the dehydrated group without permitting substantial new xylem formation. Following that recovery period, all trees were dehydrated by withholding irrigation to estimate the effect of repeated droughts on the trees. Leaf gas exchange was measured before the onset of the first drought, at the peak of the first drought, and before the onset of the second drought. In order to overcome differences in the dehydration rate among pots, all potted trees were weighed twice a week during the first drought and supplementary irrigation was given according to the slowest-drying tree. The trees were also weighed once per
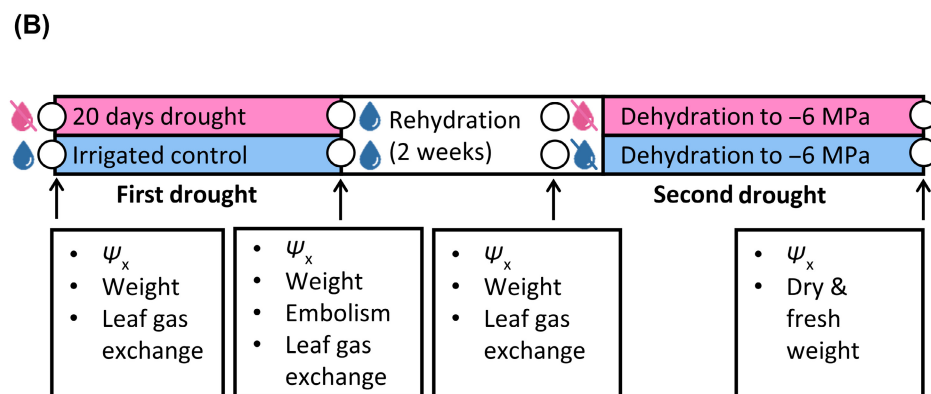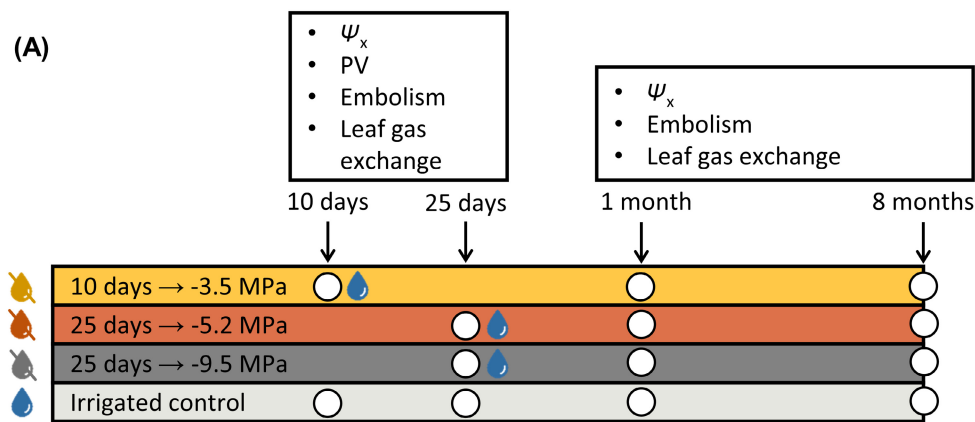
FIGURE 1   The experimental layout of the two experiments described in Sections 2.1 (A) and 2.2 (B). Colors correspond to the different treatments in each experiment, as they are presented in the other figures. White circles represent campaign days, with the measurements obtained at each campaign attached. Droplets with and without a crossing line represent irrigation and irrigation halt, respectively. Weekly monitoring is not shown here but is mentioned in the main text where relevant.

week until 3 weeks into the second drought, but without receiving supplementary irrigation. During the second drought, Ψ_x was measured weekly to monitor drought progress. Upon arriving at fatal Ψ_x (i.e., −7 MPa), tree biomass was sampled and sorted into its components (roots, stem, branches, and needles), including the pots and potting soil, dehydrated for 2 days under 60°C and dry mass was documented per tissue. See Figure 1 for a schematic description of the experimental layout, and the following sections for detailed description of the measurements.

2.3 | Leaf gas exchange and water potential

In both experiments described above, leaf gas exchange was measured using an infra-red gas analyzer system (GFS-3000, Walz). Photosynthetic photon flux density (PPFD) and chamber temperature were adjusted to ambient conditions and CO_2 levels in the chamber were set to 400 ppm. For each individual, ∼10 intact needle pairs were inserted into the sample chamber and measured after stabilization. The needle pairs were then collected, the projected surface area measured and used to derive the gas exchange per leaf area.

Ψ_x was measured on terminal branchlets containing needles that were covered for at least 30 min with tin-foil-sealed bags to allow for calibration between needles and stem water potential. The branchlets were collected from the trees at midday (12:00–14:00) and kept in the sealed bags on ice until they were measured in the lab (∼10 min). The measurements were conducted using Scholander-type (Scholander et al., 1965) pressure bomb (PMS Instrument Company).

2.4 | Embolism quantification

Embolism was quantified on terminal branchlets cut from the trees using a μCT instrument. The anatomical structure of coniferous xylem, specifically *P. halepensis*, with its relatively short tracheids (>2 mm; David-Schwartz et al., 2016), allows to quantify the embolism of cut samples without introducing artificial embolism. Figure S2 further strengthens this assumption, showing a vertical slice from a μCT of a cut branch, where air spreads just as far as 1.3 mm into the sample. Terminal intact branchlets (∼15 cm) were covered with a wet paper towel, then collected and kept in a sealed plastic bag on ice until brought to the lab (∼10 min). In the lab, the middle part of each

branchlet (∼5 cm) was covered with wax to stop water loss by dipping into heated wax (<60°C) and then instantly into ice-water. Immediately after that process, each sample was brought to the Weizmann μCT facility and scanned under 4.1 Watt, with a frame rate of 0.75 images s^{-1}, resulting in voxel size of 4.5–6 μm (RX Solutions). Following each scan, the sample was cut in the middle of the scanned area and covered with wax or with parafilm. The cut sample was scanned again, and the embolized area at the cut surface accounted for the maximum conductive area. Both scans were analyzed using ImageJ software (Schindelin et al., 2012), where the pith and bark were discarded, and air-filled area was quantified for the xylem tissue. Percent loss of conductive area (PLA) was calculated as follows:

$$PLA\,(\%) = \frac{PLA_{native}}{PLA_{cut}} \times 100, \tag{1}$$

where PLA_{native} represents the air-filled area quantified in the first scan (μm^2) and PLA_{cut} represents the air-filled area quantified in the second scan of the cut branchlet (μm^2).

To quantify the proximity of embolism to the cambium, the same scans were analyzed again, separating the xylem area (i.e., excluding the pith and the bark) into two rings, either according to annual rings (where possible) or half-way between the pith and the cambium. Embolism was quantified according to Equation (1) for each of the rings.

2.5 | Pressure–volume curves

During the peak drought campaign of each treatment group, a subset of trees was randomly sampled to construct a PV curve; that is, three individuals were sampled from the −3.5 MPa treatment group and four individuals were sampled from the −5.2 MPa treatment group during their respective campaigns. In addition, seven irrigated control plants were sampled in total during these two campaigns. A terminal branchlet was cut from each tree, placed in sealed plastic bag, and brought to the lab on ice (<10 min). In the lab, the branchlets were re-cut under water, the cut ends were submerged in distilled water and the needles were covered with plastic bags to prevent water loss, while the branchlets were left to rehydrate overnight. The next day, the branchlets were left to dehydrate on the bench while we measured Ψ_x and weight sequentially every 15–60 min. Finally, the branchlets were dehydrated for 2 days at 60°C and dry mass was recorded in order to derive relative water content (RWC). Capacitance was calculated as the slope (MPa/100%) of the point before the inflection point.

2.6 | Available water estimation

The weekly measurements of pots weight (i.e., during the repeated drought experiment, see Section 2.2), along with the dry mass obtained upon the termination of the experiment, were used to estimate the available water for the trees, as following:

$$AW = FM_{plant} - DM_{plant} - DM_{soil} - M_{pot}, \tag{2}$$

where AW is available water (kg); FM_{plant} is the weight of each tree (including the soil and pot) measured instantaneously during dehydration; DM is the collective dry mass of all tree components, measured at the end of the experiment.

2.7 | Statistical analysis

All statistical analyses were done using R and RStudio (R core team, 2021; RStudio Team, 2021). To compare PLA values of dying and surviving trees, non-parametric median test was used to account for unequal variances (Conover, 1999). To test for differences in the proximity of embolism to the cambium and the effect of treatment on g_s following one-month recovery, we employed ANOVA and post-hoc TukeyHSD, after checking for heteroskedasticity and normality of the residuals. The model correlating the increase in PLA with the decrease in Ψ_x was fitted to a sigmoidal curve, using the fitModel function in R, with the following equation:

$$PLA = \frac{100}{\left(1 + \exp\left(\frac{slope}{25(\Psi_x - P_{50})}\right)\right)}, \tag{3}$$

where Ψ_x is the measured xylem water potential; P_{50} is the water potential inducing 50% embolism; and *slope* is the shape parameter.

To model the decrease in g_s with decrease in Ψ_x (i.e., stomatal closure with a decrease in water potential), g_s values were first transformed into percentages (with 100% being 77.4 mmol H$_2$O m^{-2} LA s^{-1}, the highest levels measured in this experiment), and then fitted with a sigmoidal curve using the following Equation (3), but with P_{50} representing the water potential at which g_s decreased by 50%. The parameters of slope and P_{50} were found significant for both models, with all *p*-values <0.01.

3 | RESULTS

3.1 | Embolism effect on tree survival

Embolism levels increased as peak drought Ψ_x values were more negative (Figure 2) and reached on average PLA values of 6%, 41% and 76% for −3.5, −5.2 and −9.2 MPa treatment groups, respectively (Ψ_x values also represent mean values for each treatment group. See Section 2.1 for standard errors). Following up to a month of rehydration, we did not observe any significant decrease in embolism levels (i.e., on a short time scale). After 8 months of rehydration, we performed μCT scans of surviving trees, which showed a decrease in PLA back to low levels (i.e., ∼10%). Trees that died during the recovery period had a mean embolism level of 76% ± 4% at the peak of drought, and no tree died with PLA <62% (Figure 3). Trees that survived the drought (including both −3.5 and −5.2 MPa treatment groups) had a mean embolism level of 26%, and no tree was able to
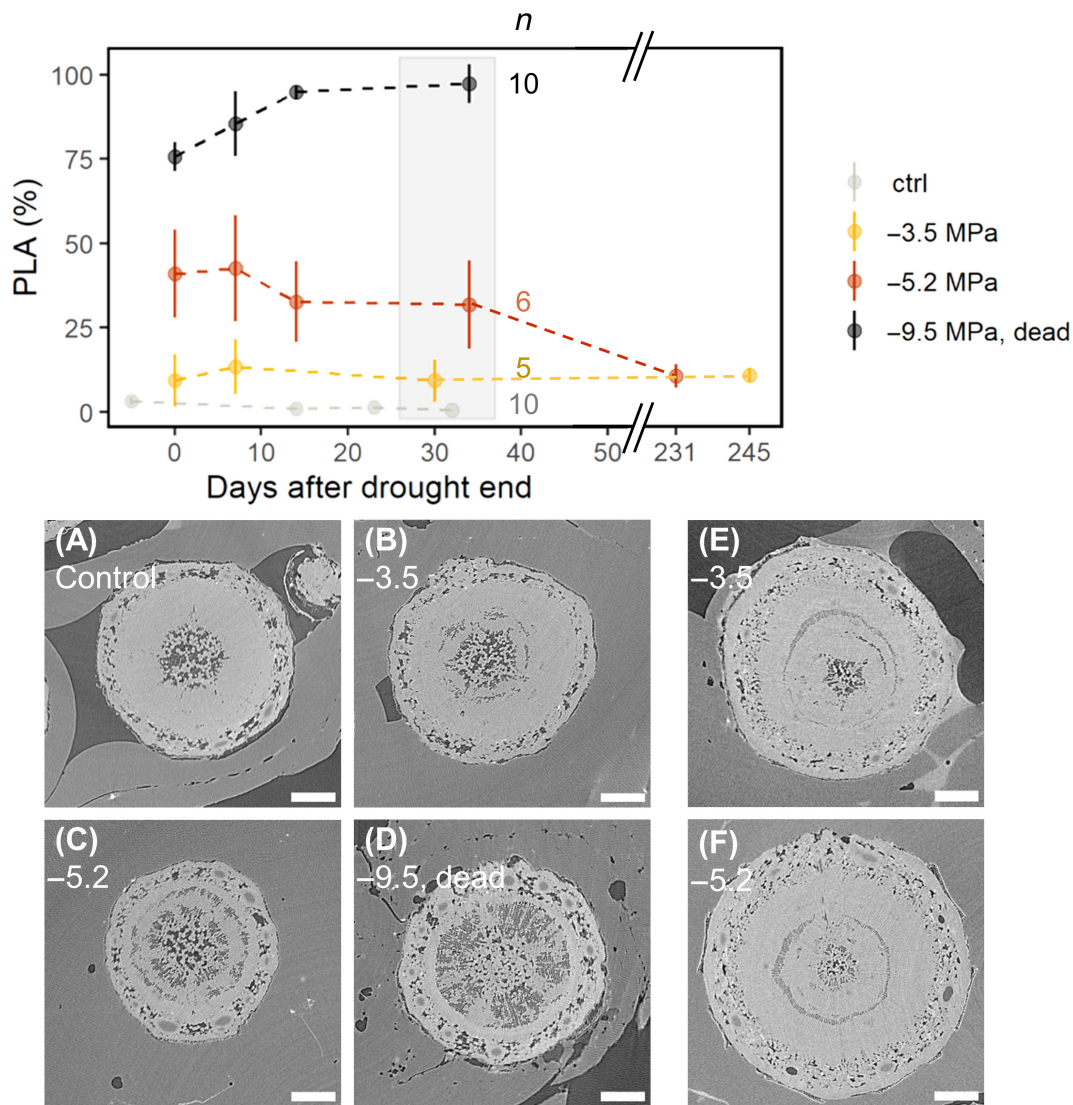
FIGURE 2 Dynamics of percent loss of conductive area (PLA) in branches of Aleppo pine saplings during 8 months of post-drought recovery. Colors represent the average PLA per drought intensity treatment. The numbers given are the number of replicates (i.e., gray = control, $n = 10$; yellow = −3.5 MPa, $n = 5$; red = −5.2 MPa, $n = 6$; black = −9.5 MPa, dead, $n = 10$. All Ψ_x values represent means of the treatment groups). Bars represent the standard error. Panels A–D and E–F show horizontal slices from µCT scans of branchlets conducted after 1 and 8 months of recovery, respectively. White bars mark 500 µm.

survive >88% embolism. We also examined the effect of embolism proximity to the vascular cambium. There were significant differences between dying and surviving trees in embolism levels of both the outer and the inner rings. However, we could not detect significant differences between the outer and inner-ring embolism for both dying and surviving trees.

3.2 | Embolism effect on tree recovery

Besides triggering mortality, hydraulic damages caused by drought can inhibit physiological recovery following drought relief. We found that Ψ_x recovered to control levels within 1 week of rehydration in all of the surviving trees and for all treatments (Figure 4). Stomatal

conductance, however, was much slower to resume. In fact, it had not fully recovered even after a month of rehydration, at which the degree of recovery was negatively proportional to the embolism level inflicted by drought. A recovery of 8 months, however, showed that trees were able to regain further functioning, and g_s did not differ between surviving trees and the control.

3.3 | Repeated droughts effect

Since drought-induced hydraulic impairment can have longer-term effects on physiological activity, it was reasonable to assume that trees with drought in their life history would respond differently to a second drought compared to trees that have never experienced
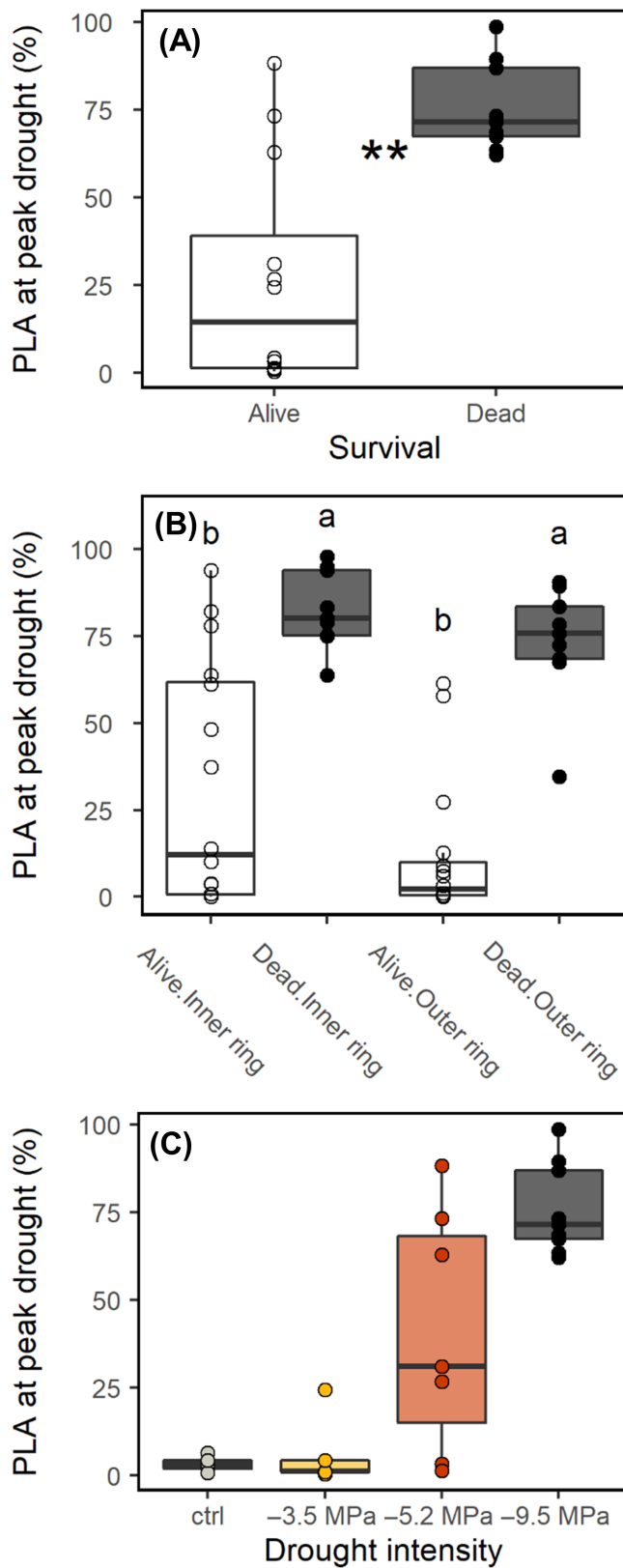
FIGURE 3 Percent loss of conductive area (PLA) at the peak of drought. (A) PLA of surviving and dying trees; (B) PLA in surviving versus dying trees separated into PLA occurring in the outer- or inner-ring, that is, adjacent or further away from the cambium; (C) PLA in the different treatments. Boxplots show the median, second and third quartiles. Each point represents data from an individual tree.

drought. We found that drought treatments from both intensities (i.e., −3.5 and −5.2 MPa) resulted in a decreased capacitance of the branchlets compared to the control trees (Figure 5). This means that the decline in Ψ_x per given decrease in branchlet RWC was larger for trees that had experienced drought before than those that did not. Interestingly, some trees that were exposed to −3.5 MPa (and not −5.2 MPa) showed a more negative slope.

Despite this lower capacitance, the trees that were subjected to a second drought showed an overall slower decrease in Ψ_x compared with trees that experienced drought for the first time, combined with a lower g_s at the onset of the second drought (Figure 6C). Notably, we found trees that were dehydrated during the first drought period to recover Ψ_x, but not g_s by the onset of the second drought. This may have allowed these trees a slower depletion of the soil available water during the second drought (Figure 6A). In addition, the dry mass of needles measured at the end of the second drought was smaller in trees that had experienced two drought periods compared to trees that had experienced one drought period (Figure 6D).

3.4 | Empiric vulnerability curve

Measured values of PLA, g_s and Ψ_x along the experiment can be constructed into a vulnerability curve, which describes the physiological processes of stomatal closure and embolism build-up along a gradual decrease in xylem tension. Such a curve is shown in Figure 7, where stomatal closure (i.e., a decrease of 90% in g_s) and P_{50} were estimated at midday Ψ_x values of −2.6 and −5.4 MPa, respectively. These values confer hydraulic safety margins (i.e., the gap between stomatal closure to P_{50}) of 2.8 MPa.

4 | DISCUSSION

Our results demonstrate the consequences of drought-induced embolism in a Mediterranean pine species. While high levels of xylem embolism may not be fatal to Aleppo pines, the trees' ability to recover gas exchange might be limited. Such an effect is disadvantageous with respect to the trees' carbon budget, but it also decreases water loss, which may potentially mitigate recurring droughts.

4.1 | Embolism effect on survival

In this study, we show that Aleppo pine trees that died following drought had lost, on average, 76% of xylem conductive tissue to embolism, and that no tree died with <62% embolism (Figure 3). These values are in the range of mortality-threshold values suggested in the literature for conifers (Adams et al., 2017; Hammond et al., 2019), and specifically for Aleppo pine (García de la Serrana et al., 2015). Yet, we acknowledge the possibility that the embolism level measured at the peak of drought in dying trees is not necessarily the mortality threshold. That is, it is possible the trees were already on a mortality trajectory at
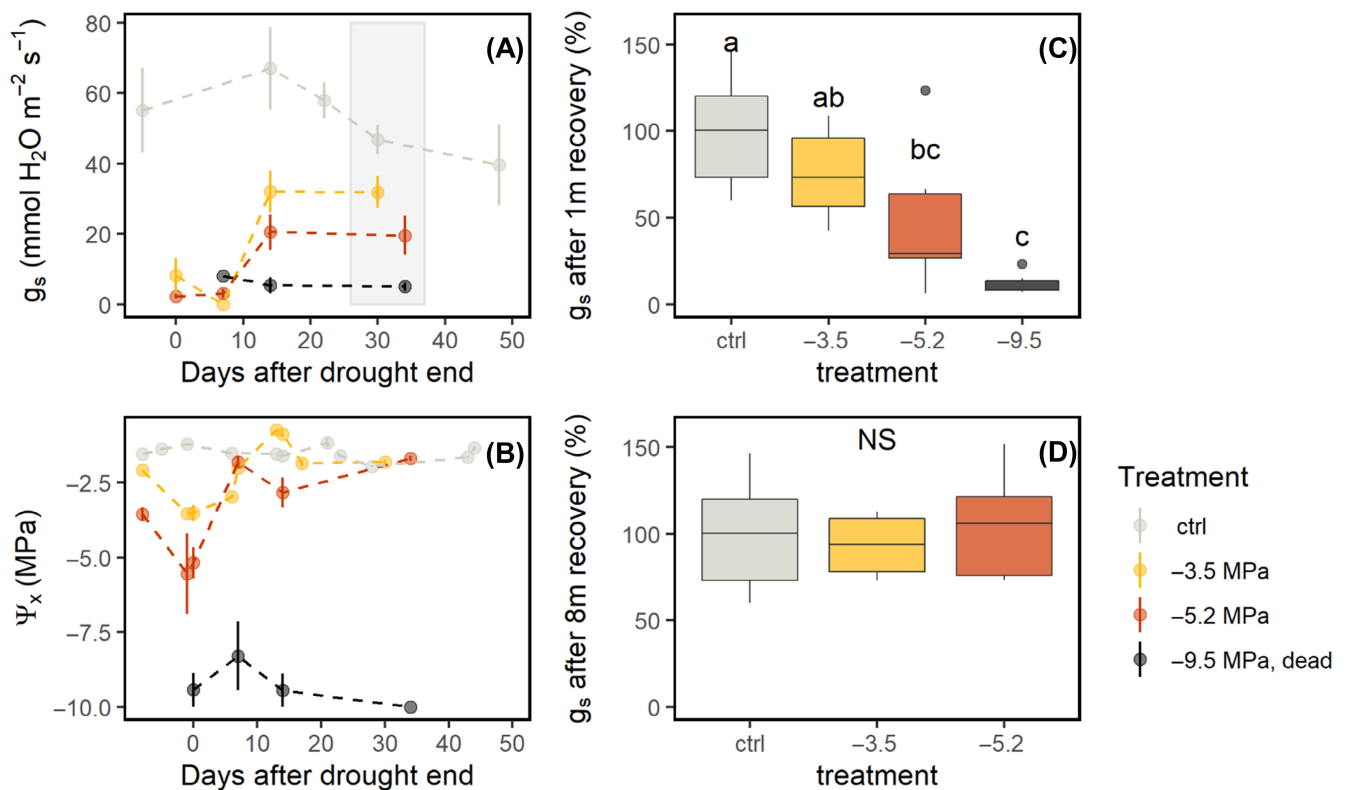
FIGURE 4 Dynamics of stomatal conductance (g_s; A) and midday xylem water potential (Ψ_x; B) during 1 month of drought recovery. Bars represent standard errors. Percent loss of g_s recovery compared to the control following 1 (C) and 8 (D) months of recovery. Boxplots show the median, second and third quartiles. Colors represent the different treatments (gray = control, $n = 10$; yellow = −3.5 MPa, $n = 5$; red = −5.2 MPa, $n = 6$; black = −9.5 MPa, $n = 10$).
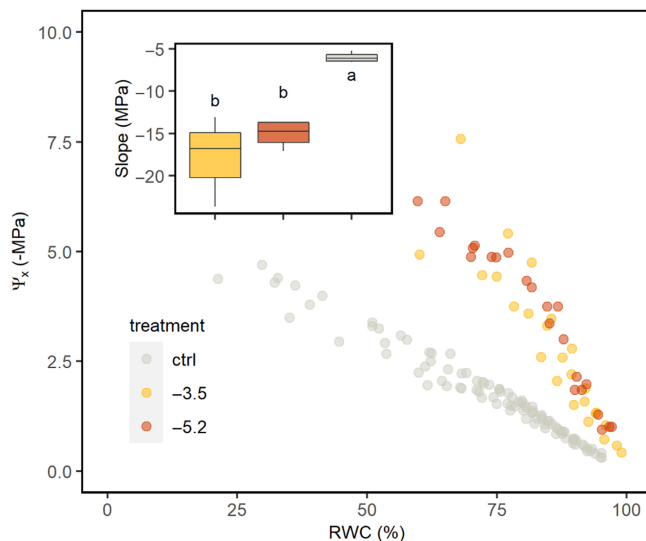

FIGURE 5 Pressure volume curves conducted for each of the treatment groups (including irrigated control) at the peak of drought. Colors represent the different treatments (gray = control, $n = 7$; yellow = −3.5 MPa, $n = 3$; red = −5.2 MPa, $n = 4$). The insert shows the slope of the trend line for the above inflection part of each curve. Boxplots represent the median, the second and third quartiles.

an earlier stage of the drought and that we, hence, may have overestimated the mean levels of embolism in dying trees.

Among trees that survived the drought, we did not detect any decrease in embolism within 1 month of rehydration. Instead, only after 8 months, embolism decreased to about pre-drought levels in surviving trees. This suggests that the recovery of hydraulic conductance occurred via long-term processes, that is, the production of new xylem conduits, rather than via rapid processes, such as embolism refilling (e.g., Rehschuh et al., 2020). This conclusion is also supported by the spatial distribution of embolism in the eight-month-rehydration μCT scans (Figure 2E,F), where embolism was constrained to the inner ring, while a new xylem with practically no embolism could be clearly identified at the outer rim. A similar finding was reported by Hammond et al. (2019), who used functional xylem staining in drought-surviving Loblolly pine trees. These results are also consistent with other recent studies, which found no evidence for rapid xylem refilling in potted Scots pine trees (Rehschuh et al., 2020) nor in mature Aleppo pine trees (Wagner et al., 2022). Yet it is worth mentioning that while μCT provides a direct observation of embolism, it does not account for hydraulic conductivity per se, and the two parameters are not necessarily identical in all cases.

In this study, we have also examined the effect of the proximity of embolism to the vascular cambium on drought survival. It was previously suggested that the fatal aspect of drought is not necessarily embolism per se but rather the impairment of water supply to vital living tissues (Körner, 2019; Mantova et al., 2021). We, therefore,
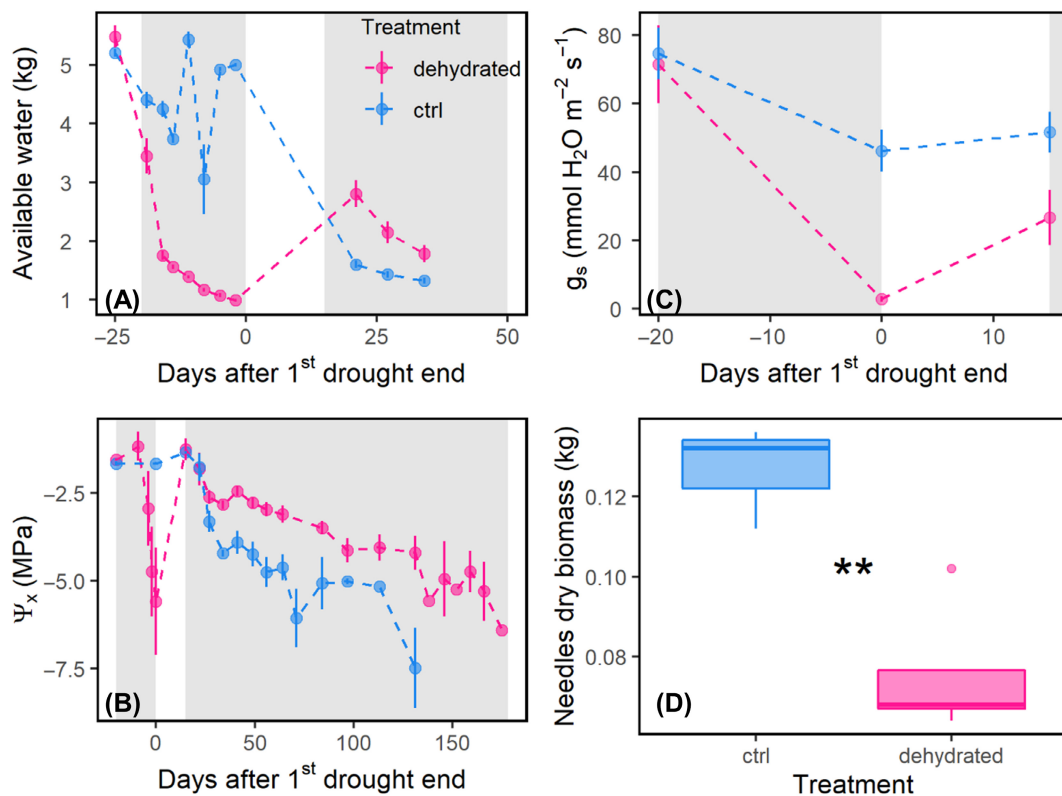
FIGURE 6 The dynamics of the available water (A) and water potential (B) during first and second drought periods. Stomatal conductance (g_s, C) during and after first drought (when drought was imposed only for the dehydrated trees). Needles dry biomass (D) at the end of the second drought. Colors represent the two treatment groups (i.e., blue = trees that were dehydrated once; $n = 5$, pink = trees that were dehydrated twice, $n = 5$). Bars represent standard errors. Shaded areas mark the two drought periods. Boxplots show the median, second and third quartiles.
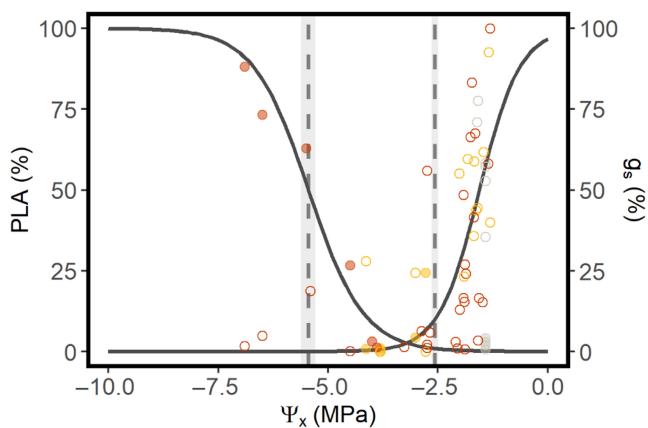

FIGURE 7 Change in percent loss of conductive area (PLA, filled circles) and stomatal conductance (g_s, open circles) with xylem water potential (Ψ_x) for Aleppo pine. Colors represent the different treatments (gray = control, $n = 10$; yellow = −3.5 MPa, $n = 5$; red = −5.2 MPa, $n = 6$). The first dashed line from the right marks the point of stomatal closure (10% of max g_s) and the second dashed line marks 50% PLA. Shaded areas are ±SE.

hypothesized that embolism in the outer part of the xylem, which potentially impairs water flow to the vascular cambium (termed here "outer-ring" embolism), would have a larger impact on survival than embolism in "old" xylem (termed here "inner-ring" embolism). In practice, we found no significant differences between levels of "outer-ring" and "inner-ring" embolism in surviving or in dying trees (Figure 3B). Bulk flow in conifers is known to be rather radially homogeneous (Cohen et al., 2008), which would readily enable the compensation for the loss of conduits that are adjacent to the cambium. A larger sample size and a wider variability in embolism levels might help answer these questions in a more precise way.

4.2 | Embolism effect on recovery

The concept of "no need for pipes when the well is dry" (Körner, 2019) may very well hold during drought. But what happens when the well is re-filled? Hammond (2020) suggested that drought-induced hydraulic damage may inhibit post-drought recovery via reduced water supply to the recovering tissues. In a recent review, it has been postulated that the rate of recovery directly relates to the degree of xylem embolism and tissue damage (Ruehr et al., 2019). There are quite a few studies demonstrating the relationship between hydraulic conductance and stomatal activity (Addington et al., 2004; Brodribb et al., 2010; Irvine et al., 1998; Rehschuh et al., 2020; Resco et al., 2009; Skelton et al., 2017). Similarly, we show here how the leaf-level g_s was reduced despite drought release, Ψ_x recovery and the existence of ample soil water to sustain transpiration. In contrast,

a recent study found that reduced branch-level water use in drought-recovering mature beech trees was the consequence of reduced foliage rather than leaf-level stomatal response (Arend et al., 2022). In our study, we have not measured leaf area in the context of drought recovery, and thus cannot assert conclusively whether there was a decrease in canopy size in our case, in addition to the decreased leaf-level g_s. However, qualitative observations (Figure S2) showed that some needle-mortality was detected in dying trees during the 1- and 2-weeks recovery campaigns (rather than the peak-of-drought campaign), but not in ones that survived. In the repeated drought recovery, we did observe a decreased needle biomass for the "two droughts" trees compared to "one drought" trees (Figure 6D), but this was measured when the trees reached fatal Ψ_x rather than during recovery. Another explanation for the unaffected leaf-level g_s in Arend et al. (2022) is the deciduous nature of beech canopy. In fact, the leaves that were measured by Arend et al. (2022) in the year following the drought were new leaves, which essentially did not experience the drought. In line with that notion, Song et al. (2022) found for a wide range of conifer species, that needle lifespan was the strongest explanatory variable for drought resilience. Most likely, the tough Aleppo pine needles, which are designed to prevail for 3–4 years, require high carbon investment and are thus not easily shed or replaced, an idea that is nicely described in a conceptual model by Zweifel and Sterck (2018).

4.3 | Repeated drought effect

Due to the long-lasting responses to drought we found in our trees, it was reasonable to assume that such responses would play a role in the survival of the trees during a repeated-droughts scenario. In deciduous plants, leaf-shedding is a common strategy during drought to minimize water loss and embolism spread (e.g., Hochberg et al., 2017), and was also observed in a pine species under mild drought stress (Nadal-Sala et al., 2021). While this does not seem to be the case with our conifer species (Figure S2), a decrease in g_s, as shown here (Figures 4 and 6C), should enable trees to survive with less water, or rather prolonging the time to fatal dehydration on a given amount of soil water. The concept of "time to hydraulic failure" was elegantly described in a trait-based model (Blackman et al., 2016, 2019), although their work focused on post-stomatal closure dehydration, linking traits like internal water storage and residual transpiration to dehydration time. Differently, in the current experiment, the dramatic difference in dehydration time between the first- and second-drought trees stemmed primarily from the rate at which the available soil water was depleted, due to differences in g_s. It is important to mention that most of the soil available water—at least in potted plants—is consumed before any signs of stress (Allen et al., 1998). This results from the retention curve of most soils, which release large parts of their stored water under very mild tensions (<0.01 MPa). Accordingly, differences in the maximal g_s could translate into substantial differences in the time to fatal dehydration. Along the same lines, Herrera et al. (2021) showed how container size affected the Ψ_x of dehydrating grapevine plants. In that aspect, it is important to note

that drought dynamics in the forest may differ from those presented here. Introducing the aspect of time into drought survival framework is crucial since, in many water-limited habitats, it is not just the sheer volume of precipitation that limits plant growth and survival but also the distribution of precipitation events (Klein et al., 2014; Raz-Yaseef et al., 2010). Thus, repeated drought events can pose a threat to plants in such ecosystems even during years that are not considered drought years; and increased time to hydraulic failure may therefore be the tipping point between survival and mortality. In that perspective, perhaps the reduced stomatal conductance presented in the previous section as impaired drought recovery can alternatively be viewed as an acclimation or even adjustment response, preparing the tree to use less water in order to increase chances to survive the next drought event.

Moreover, the longer time to reach fatal dehydration did not stem from higher capacitance (Figure 5). In fact, as was found in other studies (Barzilai et al., 2021), the drought-adjusted trees had lower capacitance, which enabled them to maintain a higher RWC (and consequently lower cell dehydration) under a given decrease in water potential. In other words, cells of drought-adjusted trees released less water per a given decrease in xylem pressure compared to non-adjusted ones, thus maintaining a better hydration status as drought progressed. This means that differences between the two treatments have indeed originated from the difference in max g_s, which controls available soil water prior to stomatal closure, rather than from changes in the internal water reservoir (which is a dominant factor post-stomatal closure). However, further research is needed to determine whether such strategy actually increases the time to hydraulic failure in a forest set-up since preservation of soil water would not necessarily be beneficial if neighboring trees that compete for the same water continue to transpire longer into a drought event and deplete the available water.

4.4 | Empiric vulnerability curve

The vulnerability curve we show in Figure 6 represents an "empiric" curve, meaning it is constructed from Ψ_x and PLA values that were measured simultaneously on naturally dehydrating intact plants rather than cut branches, similar to what was done in Paudel et al. (2020) and Gerbi et al. (2022). The P_{50} (i.e., the Ψ_x in which 50% of the xylem is embolized) was similar (<0.5 MPa difference) to the P_{50} that was found for multiple populations of Aleppo pine from Israel (David-Schwartz et al., 2016), France (Davi et al., 2020), or Spain (Morcillo et al., 2022) using the Cavitron, meaning that the fast method (Cavitron) provides functional data that is relevant for naturally dehydrating trees. Strangely, when we tested the same trees using the Cavitron or optical vulnerability and μCT for bench dehydrating shoots, they were found to be less resistant in ~1 MPa (Feng et al., 2023). One possible explanation is the seasonal change in P_{50} reported for this species (Feng et al., 2023). Feng et al. (2023) measured it in spring, while the current experiment was conducted in peak summer. Yet, we acknowledge that the small sample size may have decreased the robustness of these results.

5 | CONCLUSION

Young Aleppo pine trees showed high tolerance to embolism (risk of mortality only at ~70% embolism). While not necessarily fatal, drought-induced hydraulic damage imposed impaired stomatal activity long after drought release and prevented full recovery from drought. In contrast, lower water use was advantageous under a consecutive drought event by allowing prolonged time to fatal dehydration.

AUTHOR CONTRIBUTIONS

Yael Wagner, Uri Hochberg and Tamir Klein designed the experiments with contributions from all authors. Yael Wagner and Mila Volkov collected the data. Yael Wagner wrote the manuscript and constructed the figures with contributions from all authors.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data related with this paper are included in the figures and supplementary materials and will be uploaded to a data sharing platform after acceptance for publication.

ORCID

Yael Wagner https://orcid.org/0000-0002-2588-9278
Uri Hochberg https://orcid.org/0000-0002-7649-7004
Tamir Klein https://orcid.org/0000-0002-3882-8845

REFERENCES

Adams, H.D., Zeppel, M.J.B., Anderegg, W.R.L., Hartmann, H., Landhäusser, S.M., Tissue, D.T. et al. (2017) A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nature Ecology & Evolution, 1, 1285–1291.

Addington, R.N., Mitchell, R.J., Oren, R. & Donovan, L.A. (2004) Stomatal sensitivity to vapor pressure deficit and its relationship to hydraulic conductance in Pinus palustris. Tree Physiology, 24, 561–569.

Allen, R.G., Pereira, L.S., Raes, D. & Smith, M. (1998) Crop evapotranspiration-guidelines for computing crop water requirements-FAO irrigation and drainage - paper 56.

Arend, M., Link, R.M., Zahnd, C., Hoch, G., Schuldt, B. & Kahmen, A. (2022) Lack of hydraulic recovery as a cause of post-drought foliage reduction and canopy decline in European beech. New Phytologist, 234, 1195–1205.

Barigah, T.S., Charrier, O., Douris, M., Bonhomme, M., Herbette, S., Améglio, T. et al. (2013) Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. Annals of Botany, 112, 1431–1437.

Barzilai, O., Dag, A., Avraham, M., Zemah, H., Sorek, Y. & Hochberg, U. (2021) Productivity vs drought adaptation in olives: comparison of water relations in a modern versus a traditional cultivar. Physiologia Plantarum, 137, 2298–2306.

Blackman, C.J., Creek, D., Maier, C., Aspinwall, M.J., Drake, J.E., Pfautsch, S. et al. (2019) Drought response strategies and hydraulic traits contribute to mechanistic understanding of plant dry-down to hydraulic failure. Tree Physiology, 39, 910–924.

Blackman, C.J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S.M. & Duursma, R.A. (2016) Toward an index of desiccation time to tree mortality under drought. Plant, Cell & Environment, 39, 2342–2345.

Bodo, A.V. & Arain, M.A. (2021) Radial variations in xylem sap flux in a temperate red pine plantation forest. Ecological Processes, 10, 1–9.

Brodribb, T.J., Bowman, D.J.M.S., Nichols, S., Delzon, S. & Burlett, R. (2010) Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. New Phytologist, 188, 533–542.

Choat, B., Brodribb, T.J., Brodersen, C.R., Duursma, R.A., López, R. & Medlyn, B.E. (2018) Triggers of tree mortality under drought. Nature, 558, 531–539.

Cochard, H. & Delzon, S. (2013) Hydraulic failure and repair are not routine in trees. Annals of Forest Science, 70, 659–661.

Cohen, Y., Cohen, S., Cantuarias-Aviles, T. & Schiller, G. (2008) Variations in the radial gradient of sap velocity in trunks of forest and fruit trees. Plant and Soil, 305, 49–59.

Conover, W.J. (1999) Practical nonparametric statistics. New York, NY: John Wiley & Sons, Inc.

Davi, H., Durand-Gillmann, M., Damesin, C., Delzon, S., Petit, C., Rozenberg, P. et al. (2020) Distribution of endemic bark beetle attacks and their physiological consequences on Pinus halepensis.

David-Schwartz, R., Paudel, I., Mizrachi, M., Delzon, S., Cochard, H., Lukyanov, V. et al. (2016) Indirect evidence for genetic differentiation in vulnerability to embolism in Pinus halepensis. Frontiers in Plant Science, 7, 768.

Feng, F., Wagner, Y., Klein, T. & Hochberg, U. (2023) Xylem resistance to cavitation increases during summer in Pinus halepensis. Plant Cell and Environment. Available from: https://doi.org/10.1111/PCE.14573

García de la Serrana, R., Vilagrosa, A. & Alloza, J.A. (2015) Pine mortality in Southeast Spain after an extreme dry and warm year: interactions among drought stress, carbohydrates and bark beetle attack. Trees—Structure and Function, 29, 1791–1804.

Gerbi, H., Paudel, I., Zisovich, A., Sapir, G., Ben-Dor, S. & Klein, T. (2022) Physiological drought resistance mechanisms in wild species vs. rootstocks of almond and plum. Trees—Structure and Function, 36, 669–683.

Hammond, W.M. (2020) A matter of life and death: alternative stable states in trees, from xylem to ecosystems. Frontiers in Forests and Global Change, 3, 135.

Hammond, W.M., Yu, K., Wilson, L.A., Will, R.E., Anderegg, W.R.L. & Adams, H.D. (2019) Dead or dying? Quantifying the point of no return from hydraulic failure in drought-induced tree mortality. New Phytologist, 223, 1834–1843.

Herrera, J.C., Savi, T., Mattocks, J., De Berardinis, F., Scheffknecht, S., Hietz, P. et al. (2021) Container volume affects drought experiments in grapevines: insights on xylem anatomy and time of dehydration. Physiologia Plantarum, 173, 2181–2190.

Hochberg, U., Windt, C.W., Ponomarenko, A., Zhang, Y.-J., Gersony, J., Rockwell, F.E. et al. (2017) Stomatal closure, basal leaf embolism and shedding protect the hydraulic integrity of grape stems. Plant Physiology, 174, 764–775.

Irvine, J., Perks, M.P., Magnani, F. & Grace, J. (1998) The response of Pinus sylvestris to drought: stomatal control of transpiration and hydraulic conductance. Tree Physiology, 18, 393–402.

Kannenberg, S.A., Schwalm, C.R. & Anderegg, W.R.L. (2020) Ghosts of the past: how drought legacy effects shape forest functioning and carbon cycling (M Rejmanek, Ed.). Ecology Letters, 23, 891–901.

Klein, T., Rotenberg, E., Cohen-Hilaleh, E., Raz-Yaseef, N., Tatarinov, F., Preisler, Y. et al. (2014) Quantifying transpirable soil water and its relations to tree water use dynamics in a water-limited pine forest. *Ecohydrology*, 7, 409–419.

Körner, C. (2019) No need for pipes when the well is dry—a comment on hydraulic failure in trees. *Tree Physiology*, 39, 695–700.

Mantova, M., Herbette, S., Cochard, H. & Torres-Ruiz, J.M. (2021) Hydraulic failure and tree mortality: from correlation to causation. *Trends in Plant Science*, 27, 335–345.

Morcillo, L., Muñoz-Rengifo, J.C., Torres-Ruiz, J.M., Delzon, S., Moutahir, H. & Vilagrosa, A. (2022) Post-drought conditions and hydraulic dysfunction determine tree resilience and mortality across Mediterranean Aleppo pine (Pinus halepensis) populations after an extreme drought event. *Tree Physiology*, 42(7), 1364–1376. Available from: https://doi.org/10.1093/TREEPHYS/TPAC001

Nadal-Sala, D., Grote, R., Birami, B., Knüver, T., Rehschuh, R., Schwarz, S. et al. (2021) Leaf shedding and non-stomatal limitations of photosynthesis mitigate hydraulic conductance losses in Scots pine saplings during severe drought stress. *Frontiers in Plant Science*, 12, 715127.

Paudel, I., Gerbi, H., Wagner, Y., Zisovich, A., Sapir, G., Brumfeld, V. et al. (2020) Drought tolerance of wild versus cultivated tree species of almond and plum in the field. *Tree Physiology*, 40, 454–466.

Phillips, N., Oren, R. & Zimmermann, R. (1996) Radial patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. *Plant, Cell and Environment*, 19, 983–990.

R Core Team. (2021) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raz-Yaseef, N., Yakir, D., Rotenberg, E., Schiller, G. & Cohen, S. (2010) Ecohydrology of a semi-arid forest: partitioning among water balance components and its implications for predicted precipitation changes. *Ecohydrology*, 3, 143–154.

Rehschuh, R., Cecilia, A., Zuber, M., Faragó, T., Baumbach, T., Hartmann, H. et al. (2020) Drought-induced xylem embolism limits the recovery of leaf gas exchange in Scots pine. *Plant Physiology*, 184, 852–864.

Resco, V., Ewers, B.E., Sun, W., Huxman, T.E., Weltzin, J.F. & Williams, D.G. (2009) Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C3 woody legume, *Prosopis velutina*. *New Phytologist*, 181, 672–682.

RStudio Team. (2021) *RStudio: integrated development environment for R*. Boston, MA: RStudio, PBC.

Ruehr, N.K., Grote, R., Mayr, S. & Arneth, A. (2019) Beyond the extreme: recovery of carbon and water relations in woody plants following heat and drought stress. *Tree Physiology*, 39, 1285–1299.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T. et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9, 676–682.

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A. & Hammel, H.T. (1965) Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. *Science (New York, NY)*, 148, 339–346.

Skelton, R.P., Brodribb, T.J., McAdam, S.A.M. & Mitchell, P.J. (2017) Gas exchange recovery following natural drought is rapid unless limited by loss of leaf hydraulic conductance: evidence from an evergreen woodland. *New Phytologist*, 215, 1399–1412.

Song, Y., Sterck, F., Zhou, X., Liu, Q., Kruijt, B. & Poorter, L. (2022) Drought resilience of conifer species is driven by leaf lifespan but not by hydraulic traits. *New Phytologist*, 235, 978–992.

Wagner, Y., Feng, F., Yakir, D., Klein, T. & Hochberg, U. (2022) In situ, direct observation of seasonal embolism dynamics in Aleppo pine trees growing on the dry edge of their distribution. *New Phytologist*, 235, 1344–1350.

Zimmermann, M.H. (1983) *Xylem structure and the ascent of sap*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Zweifel, R. & Sterck, F. (2018) A conceptual tree model explaining legacy effects on stem growth. *Frontiers in Forests and Global Change*, 1, 9.

SUPPORTING INFORMATION

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