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To -8 MPa and back: in-situ annual water relation in non-irrigated olive trees

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Abstract

Olive trees are known for their capability to endure and recover from low stem water potentials (Ψ_{stem} < -6 MPa) and even maintain substantial gas exchange under such conditions. In the current experiment, we tested the physiological responses of mature olive trees in-situ while enduring such Ψ_{stem} under field conditions for seven months of rainless Israeli summer. To characterize olive trees' seasonal response to drought, we followed the annual patterns (a measurement day once a month) of Ψ_{stem}, gas exchange, osmotic adjustment, and hydraulic conductance of mature bearing olive trees that were disconnected from irrigation. We found that through the dry summer, the trees gradually dehydrated to a minimum of -8.1 MPa in October. An increase of 1320 mmol/kg in the leaf osmotic concentration through the summer was part of the tree's acclimation to the low Ψ_{stem}. Surprisingly, despite the low Ψ_{stem} that have probably led to substantial xylem cavitation, the trees maintained stomatal conductance of at least 0.023 mol m^{-2} s^{-1} and positive assimilation throughout the year. Following the winter rains, the plants recovered their Ψ_{stem}, gas exchange, and hydraulic conductance to maximal values. The results demonstrate olives' remarkable ability to tolerate Ψ_{stem} while maintaining above minimal gas exchange and producing fruits.

Introduction

Olives (*Olea europaea*), perhaps the most emblematic crop of the Mediterranean (Sofo *et al.*, 2008), is one of the most important tree crops in the world. Covering 10.5 million hectares and producing 20.5 million tons, olives are a market of 20 billion dollars per year (FAO stat 2018). Most of the production areas are located around the Mediterranean basin, with Spain, Italy, Greece, Tunisia, and Turkey being the top producers (IOC2020). Despite the typical rainless summer in these production areas, the large majority of olive orchards are grown without irrigation (77% based on the IOC survey from 2016-2017). The inability to irrigate, combined with the climate change processes that are threatening most of the Mediterranean (Nasrallah and Balling, 1995; Lionello *et al.*, 2014), emphasizes that understanding olives responses to drought is of critical importance.

Olives are considered a drought-tolerant crop that is capable of sustaining low water potential (Ψ_{stem}) while maintaining positive assimilation (Sofo *et al.*, 2008; Fernández, 2014). Under typical Mediterranean environmental conditions, when mature olive trees are grown in non irrigated orchards, they dehydrate to stem water potential of -3 to -3.5 MPa. These Ψ_{stem} values allow them to maintain substantial gas exchange, which is critical for the oil accumulation in the fruit toward the end of the dry season (Giorio *et al.*, 1999; Tognetti *et al.*, 2007; Kitsaki and Drossopoulos, 2005; Diaz-Espejo *et al.*, 2007). When orchards were dried to lower Ψ_{stem} ($-5 < \Psi_{stem} < -4$ MPa), stomatal conductance was still higher than 0.05 mol m^{-2} s^{-1} (Naor *et al.*, 2013; Torres-Ruiz *et al.*, 2015). Even when olive trees were dried to $\Psi_{stem} < -6$MPa, they were able to retain pre-stress functionality upon irrigation due to the high resistance of their hydraulic system and photosynthetic apparatus (Angelopoulos *et al.*, 1996; Moriana *et al.*, 2003; Ennajeh *et al.*, 2008; Perez-Martin *et al.*, 2014). Furthermore, olive trees showed a noteworthy ability to accumulate osmolites, shifting their turgor loss point Ψ_{TLP} by more than 2 MPa through the dehydration, which allows them to maintain turgor even when dried to $\Psi_{stem} < -6$ MPa (Ennajeh *et al.*, 2008; Sofo *et al.*, 2008; Dichio *et al.*, 2006a; Dichio *et al.*, 1996). In some experiments, olives were even capable of maintaining substantial gas exchange ($g_s > 0.05$ mol m^{-2} s^{-1}) under Ψ_{stem} lower than -6 MPa (Ennajeh *et al.*, 2008; Tugendhaft *et al.*, 2016), but not in others (Angelopoulos *et al.*, 1996; Sofo *et al.*, 2004; Perez-Martin *et al.*, 2014). Based on such findings, it appears that even if olives will dry to significantly lower Ψ_{stem} than those that they typically experience under non-irrigated conditions, they will not suffer irreversible damage and possibly might even be able to maintain assimilation. Having said that, nearly all of the experiments in which olives were dried to $\Psi_{stem} < -6$ MPa were conducted on potted trees (except for Moriana *et al.*, 2003) and the seasonal dynamic of gas exchange and osmotic adjustment of mature bearing trees under such stress is still vague.

There are major differences between short dehydration of trees in pots versus field-grown trees under a natural drought that might result in different outcomes for the trees. On the one hand, the length of a whole summer drought accompanied with high ET in that period (more than six months in a typical Israeli summer) might present a bigger challenge than a short exposure to low Ψ_{stem}. For example, sustaining low assimilation through an entire summer might deplete the tree's carbon storage that is required for osmotic adjustment and respiration (Hasibeder *et al.*, 2015). On the other hand, field-grown plants normally have substantial root volume with a larger water holding capacity, which results in slower dehydration. Accordingly, field-grown plants have much more time to acclimate to the gradually intensifying drought conditions.

In the current study, we followed the water relations of mature olive trees *in-situ*. The trees were grown under rainfed conditions in Israel over the course of a year. We wanted to find how mature olives sustain the long summer drought of the region where they were first domesticated (Langgut *et al.*, 2019). The goal was to evaluate their acclimation to drought and see if the drought tolerance that olives exhibited in pots under short-term dehydration will hold under a whole summer drought. Specifically, we were curious to see which drought severity eliminates assimilation.

Materials and Methods

Experimental setup

The experiment took place between April 2019 and March 2020 and consisted of four fruit-bearing olive trees (*Olea europaea* cv. Arbequina), 20 years old, growing in the experimental farm of Volcani center (31.986, 34.823). The trees were growing in the middle of a row of 28 trees, which is the remainder of an orchard that was cut down two years prior to the experiment. Only three rows of the orchard were left, and we monitored trees from the central one. The spacing between trees in that row was two meters. The soil texture (at 30 cm depth) is sandy loam and composed of 62% sand, 25% silt and 13% clay. Soil porosity is 45%. From planting and until one year prior to our study, the orchards received irrigation of around 400 mm/year during the dry months. Irrigation had stopped a year and a half before we started our measurements, meaning that we measured the second summer with no irrigation.

Weather data was taken from the Beit Dagan meteorological weather station (https://ims.gov.il/) located 2.26 km from the experimental site. The winter before the measurements had started (winter of 2018-2019) had 703 mm, which is 38.5% higher than the 2008-2018 average for the area (507.9 mm). In the year in which the experiment took place, precipitation was 684 mm, as described in Figure S1. The annual potential evapotranspiration (ET_0) between March 2019 to March 2020 was 214% (1466.3 mm) higher than precipitation at the same time. Maximal and Minimal temperatures for that period (42.3 and 3.8 ^{O}C, respectively), as well as ET_0, are presented in Figures S1 and S2 and were well in the range of the last ten years (0.3 - 43.5 ^{O}C).

Field measurements

Stem water potential and gas exchange were measured once a month throughout the year (April 2019 – March 2020), at three hours intervals, five times a day, from predawn till dusk. On the same days, the leaves' osmotic content was evaluated at predawn and 16:00. The specific dates which were measured are: 3/4/2019, 5/5/2019, 3/6/2019, 2/7/2019, 4/8/2019, 29/8/2019, 3/10/2019, 4/11/2019, 2/12/2019, 6/1/2020, 5/2/2020, 30/3/2020.

Measurements were conducted only on clear days. All measurements were made on leaves that were formed in February and March 2019, meaning that they were 1-2 months old when we started the experiment and 13-14 month-old when we terminated it. To understand the effect of leaf maturation on the measured parameters, in the last time point (30th of March 2020), we measured both 1-2 and 13-14 month-old leaves.

The stem water potential (Ψ_{stem}; MPa) was measured with a pressure chamber (1505D, PMS, Albany, OR, USA) on two twigs per tree (east and west facing) at five time points a day. Twigs with 4-8 leaves were bagged with aluminum foil bags for at least 30 minutes before excision and transported to the lab for measurement. The time span between excision and measurement was up to 30 minutes, during which the leaves were double-bagged to prevent dehydration. In a pre-experiment trial, we found that maintaining double-bagged leaves for 30 minutes in room conditions resulted in less than 0.05 MPa drop.

Stomatal conductance (g_s; mmol m^{-2} s^{-1}) and net assimilation (A_N; µmol m^{-2} s^{-1}) were measured using a commercial gas exchange system (Licor6400, Lincoln, NE) on one leaf per tree at the same time as Ψ_{stem} sampling. We only measured wide sun-exposed leaves that were able to cover the 2cm^2 cuvette. The chamber parameters were set to CO_2 concentration of 400 µmol m^{-2} s^{-1} with ambient PAR, humidity, and temperature. The ambient conditions in the measurement days appear in Figs S1 and S2.

To evaluate the leaf osmotic concentration (π; mmol/kg), leaves were collected at predawn and the afternoon (15:30-17:00, depending on day length) into sealed bags and frozen within 10 minutes. At both predawn and 16:00, we averaged two samples for each tree, one containing west-facing leaves and the other containing east-facing leaves. Osmolite could accumulate passively due to dehydration of the leaf. To reveal the active osmotic adjustment and account for the leaf dehydration, another set of leaves (two for each tree) was used to measure the osmotic concentration at full hydration (π_{100}). These leaves were frozen only after their twigs were cut under water and hydrated for 10-120 minutes. By measuring every 10 min the Ψ_{stem} of other twigs collected from the same trees and going through the same hydration protocol, we made sure that the hydration time was the minimal time sufficient to achieve $\Psi_{stem} > -0.2$ MPa. This way, we made sure all leaves are near their maximal water content while avoiding potential oversaturation of the leaves. Generally, in the early spring, rehydration was short (10 min), while later in the summer, longer rehydration was required to account for the low leaf conductance. To extract the leaf sap, the leaves were thawed and centrifuged (Eppendorf 5417R, Hamburg, Germany) at 12000*g* for three minutes. The sap osmotic concentration (π) was evaluated in a vapor osmometer (VAPRO 5600, WESCOR, Puteaux, France). The leaf chemical potential ($\Psi_{\pi;}$; MPa) was calculated based on π, according to the Van't Hoff equation. Essentially, 400 mmol/kg were converted into -1 MPa.

To estimate the leaf's turgor pressure (P_{turgor}; MPa), we subtracted the chemical potential from the stem water potential. Because the stem water potential is higher than the leaf water potential, our calculation possibly overestimated P_{turgor} by a few bars. More critical for the calculation of P_{turgor} is the effect of the apoplastic fraction (A_f), which contains a very low solute content (McCully, 1999). Because the evaluation of A_f is not straightforward (Callister *et al.*, 2006), we simulated the effect of a range of A_f on the P_{turgor}, assuming the apoplastic solute concentration is 0:

$$P_{turgor} = \Psi_{stem} - \frac{\Psi_{\pi}}{1 - A_f}$$

Hydraulic conductance

The twig hydraulic conductance (K_{twig}; mmol m^{-2} s^{-1} MPa^{-1}) was measured in the second week of May 2020, using the leaf rehydration technique (Brodribb and Holbrook, 2003) on twigs with 4–8 leaves. Long branches (~1 m) were allowed to dry in the lab for up to four days to develop various Ψ_S. Before measurements, the branch was covered with a plastic bag for 30 min to ensure equilibrium. Then, one twig was used to determine the initial water potential (Ψ_I) while another was cut underwater to rehydrate. Rehydration lasted for 60-160 seconds, depending on the Ψ_I (more time for lower Ψ_I). The rehydrated leaf was covered with a plastic bag for another 30 min before its final water potential (Ψ_F) was determined. K_{twig} was calculated from the leaf rehydration kinetics (Brodribb and Holbrook, 2003):

$$K_{twig} = -\ln\left(\frac{\Psi_F}{\Psi_I}\right) \times \frac{C}{t}$$

where C is the twig capacitance, and t is the rehydration time in seconds. C (mmol m^{-2} MPa^{-1}) was measured as the average slope of the pressure-volume relationship, performed as described in (Turner, 1988). At the end of the pressure-volume analysis, the leaf area of the twigs was estimated using ImageJ to calculate their C. Different C was calculated for Ψ's that were higher or lower than the turgor loss point.

In order to evaluate the Ψ_{stem} in which 50% of K_{twig} is lost (K_{twig50}), the data were fit to an exponential sigmoidal regression using a slop coefficient (α) as follows:

$$K_{twig} = \frac{100}{1+e^{\alpha(\Psi_{stem}-K_{twig50})}}$$

The complementary conductance to K_{twig} is the hydraulic conductance between the soil and stem ($K_{s\text{-}s}$; does not include the leaf conductance; mmol m^{-2} s^{-1} MPa^{-1}). Together they represent the entire hydraulic conductance of the liquid phase in the tree. The $K_{s\text{-}s}$ for each measurement date was evaluated using Ohm's law analogy:

$$K_{s-s} = \frac{E}{\Psi_{PD} - \Psi_{stem-min}}$$

where transpiration (E) was measured with the Licor6400 (as described above) at the time the trees exhibited their lowest stem water potential ($\Psi_{stem\text{-}min}$; typically at 16:00) and divided by the gradient between the Ψ_{PD} and $\Psi_{stem\text{-}min}$.

Statistical analysis

Statistical analysis was done using RStudio environment (R Core Team 2021, RStudio Team 2021). To detect diurnal changes in physiological parameters (i.e. Ψ_{stem}, A_N, g_s; Figure. 1), each of the four examined dates was subjected to repeated measures ANOVA using the “ez” package (Lawrence, 2016), where the different trees represented the different subjects and the sampling time was considered as the within-subject variance. Then, values were subjected to Tukey HSD test using the “multcomp” package (Hothorn *et al.*, 2008). Seasonal change of physiological parameters (Figure. 2) was examined using repeated measures ANOVA, with the campaign date considered as the within-subject variance and time of sampling considered as the between-subject variance. Tukey HSD test was conducted separately for pre-dawn and afternoon measurements. Seasonal change in π and π_{100} (Figure. 3) was tested the same way, where date was considered as the within-subject variance and sampling time as the between-subject variance for both the repeated ANOVA analysis and the Tukey HSD test. The confidence level for all conducted tests was pre-determined to be 95% (i.e. $\alpha = 0.05$).

Results

Stem water potential (Ψ_{stem}) and gas exchange

The entire set of raw data and its statistical analysis are presented in supplementary file S1. Our measurements revealed that the trees dehydrated down to a Ψ_{stem} of -8.1 MPa in the end of the summer while maintaining positive assimilation. All the measured physiological parameters had a clear daily and seasonal pattern. During most of the year, all the measured parameters decreased during the day (Figure 1). The maximal daily Ψ_{stem} measured at predawn (Ψ_{PD}) or maximal daily stomatal conductance and assimilation that were measured in the morning declined to minimal values in the afternoon (between 12:00 and 18:00). This was only different in winter when the maximal g_s and A_N were exhibited at midday (Figure 1), probably because low temperatures, rather than water availability, was the limiting factor for gas exchange. The physiological status of the trees changed dramatically throughout the season, mostly as a function of time from the last effective rain (Figure 2). In early April, two days after the last substantial rain (>10 mm), the trees exhibited minimal Ψ_{stem} of -0.9 MPa, maximal g_s of 0.26 mol m^{-2} s^{-1} and maximal A_N of 16 μmol m^{-2} s^{-1}. From April 2019 onward, all measured parameters were gradually declining, reaching minimal values in October 1st (-8.1 MPa, 0.05 mol m^{-2} s^{-1}, and 4.16 μmol m^{-2} s^{-1}, for the minimal Ψ_{stem}, maximal g_s, and maximal A_N , respectively). The severe stress at peak summer is also manifested in the subcuticular CO_2 concentration which dramatically increased (Figure S3). These low values were maintained until December 2nd (-6.5 MPa, 0.05 mol m^{-2} s^{-1}, 4.9 μmol m^{-2} s^{-1}) despite a total of 25.7 mm of precipitation in the two months prior to that measurement. Following the major rains in January 2020 (363 mm), all three parameters made a sharp recovery to maximal values by February 1st 2020 (Figure 2). In agreement with the Darcy equation, as g_s and the transpiration decreased during summer, also the difference between Ψ_{PD} and the minimal Ψ_{stem} decreased from an average of 1.5 MPa in May and June to an average of 0.6 MPa between August and December (Figure 2). It's important to mention that the minimal g_s was never lower than 0.023 mol m^{-2} s^{-1} (reached in October), even when the minimal Ψ_{stem} was -8.1 MPa. It seems that under a seasonal dehydration, the Ψ_{stem} at 90% stomatal closure (an important value for species comparison; StPaul *et al.*, 2017) of olives is ca -8 MPa.

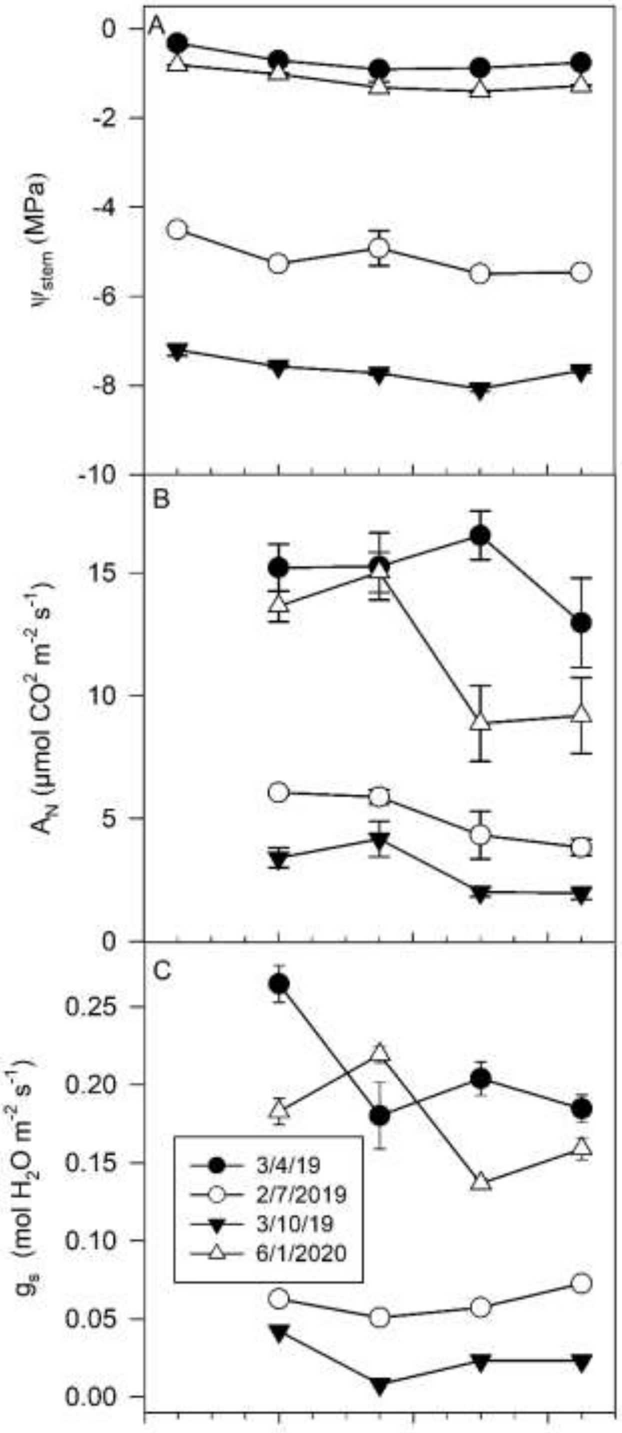

Figure 1: The daily patterns of water potential and gas exchange at different seasons.
Stem water potential (Ψ_{stem}; A) net assimilation (A_N; B) and stomatal conductance (g_s; C) as a function of daytime on four dates during the experiment. Data are means ± SE, n=4. Statistical analysis of the data is presented in supplementary file S1.

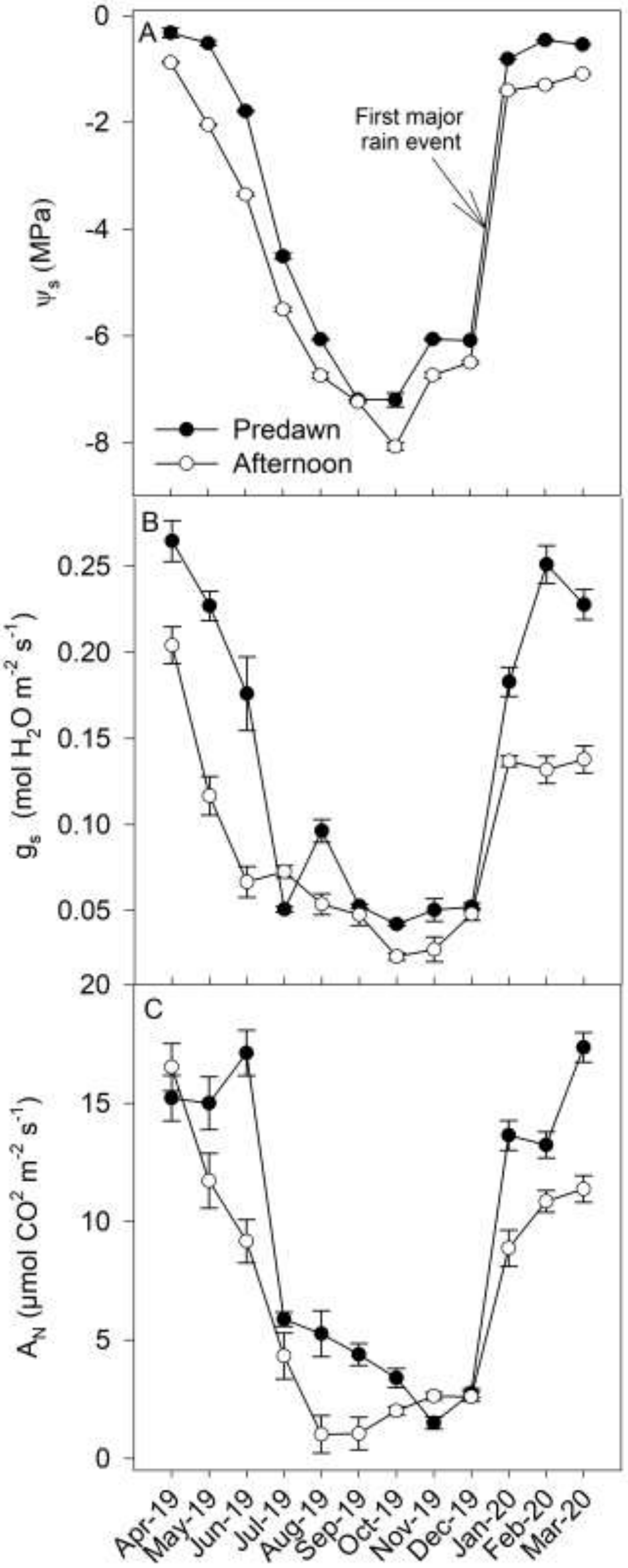

Figure 2: Annual pattern of water potential and gas exchange.
Stem water potential (Ψ_{stem}; A), Stomatal conductance (g_s; B), and Assimilation (A_N; C) measured at predawn (for Ψ_{stem}) or morning (for A_N and g_s), and the afternoon (for all parameters) along the experiment. Data are means ± SE, n=4. Statistical analysis of the data is presented in supplementary file S1.

To understand what is the actual minimal g_s that olives are capable of, we bench dehydrated 5 small branches for 24 hours under lab conditions until their Ψ_{stem} was -5.2 ± 0.06 MPa. We then measured the stomatal conductance using the Licor6400 of both current year leaves and previous year leaves. We found that the minimal g_s of 3-month-old leaves was 3 ± 0.4 mmol m^{-2} s^{-1} and of 15-month-old leaves was 5.1 ± 0.1 mmol m^{-2} s^{-1}.

Osmotic adjustments

The large reduction in Ψ_{stem} was accompanied by major osmotic adjustment (Figure 3). During summer, the leaf osmotic concentration at predawn (π_{PD}) increased from 866 mmol/kg in April 1st to 2186 and 2082 mmol/kg in August and September, respectively. When π is plotted in respect to the time of year, it goes hand in hand with the Ψ_{stem} dynamics, with one major exception. Even after the major rains of January, π_{100} at predawn was still 39% higher than its April 2019 value (1089 compared with 780 mmol/kg). This difference probably originates from leaf maturation, as young leaves in April 2020 showed similar π_{100} to the leaves sampled in April 2019 (780 compared with 858 mmol/kg, respectively). Part of the increase in π during the summer originated from the leaf dehydration. The active osmotic adjustment can be clearly viewed through the seasonal pattern of the osmotic concentration in rehydrated leaves (Figure 3B). The results suggest that most of the osmotic adjustment originated from active osmolyte accumulation. For example, the accumulation of osmotic concentration in rehydrated leaves at predawn explains 55% (754 of 1354 mmol/kg) of the total osmotic adjustment between April and August and 75% (825 of 1129 mmol/kg) of the total osmotic adjustment between April and September.

In addition to the annual osmotic adjustment, the trees also exhibited a daily osmotic adjustment, increasing by up to 578 mmol/kg between predawn and the afternoon (Fig. 3A). About half of that increase (on average 49%) could be attributed to active osmotic adjustment as exhibited by π_{100}, which increased by 93-233 mmol/kg between predawn and the afternoon (Figure 3B). Despite the assumption that the daily accumulation of osmolites should correspond to the photosynthetic rates, the active osmotic adjustment was not smaller in the dry months (103 mmol/kg between July and November) compared with the spring and early summer (93 mmol/kg between December and June).

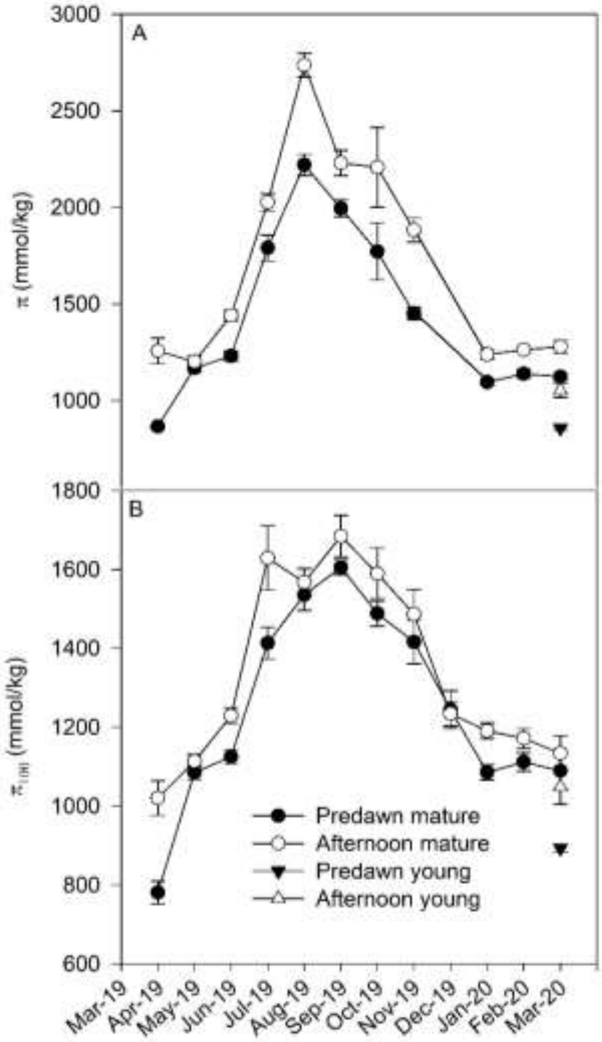

Figure 3: Annual dynamics of the leaf osmotic concentration.

The leaf native osmotic concentration (π; A) or saturated osmotic concentration (π_{100}; B) at predawn and the afternoon along the experiment. In the last measurement day (March 20th) we collected data for both mature leaves (the same type of leaves that were followed throughout the experiment) or young leaves that were formed in the same calendaric year. Data are means ± SE, n=4. Statistical analysis of the data is presented in supplementary file S1.

Hydraulic conductance

Rehydration kinetics measurements suggest that olive twigs should lose 50% of their hydraulic conductance already at -3.2MPa and 88% of their hydraulic conductance at -5.3 MPa (Figure 4A). Such data suggest that at peak summer, the leaves should have lost most of their hydraulic conductance. Measurement of the hydraulic conductance from the soil to the stem ($K_{S\text{-}S}$) (Figure 4B) suggests lower vulnerability as compared with K_{twig}, decreasing from 6.4 mmol m^{-2} s^{-1} MPa^{-1} in spring to ~2 mmol m^{-2} s^{-1} MPa^{-1} through most of the summer and exhibiting minimal values of <1 mmol m^{-2} s^{-1} MPa^{-1} in December.

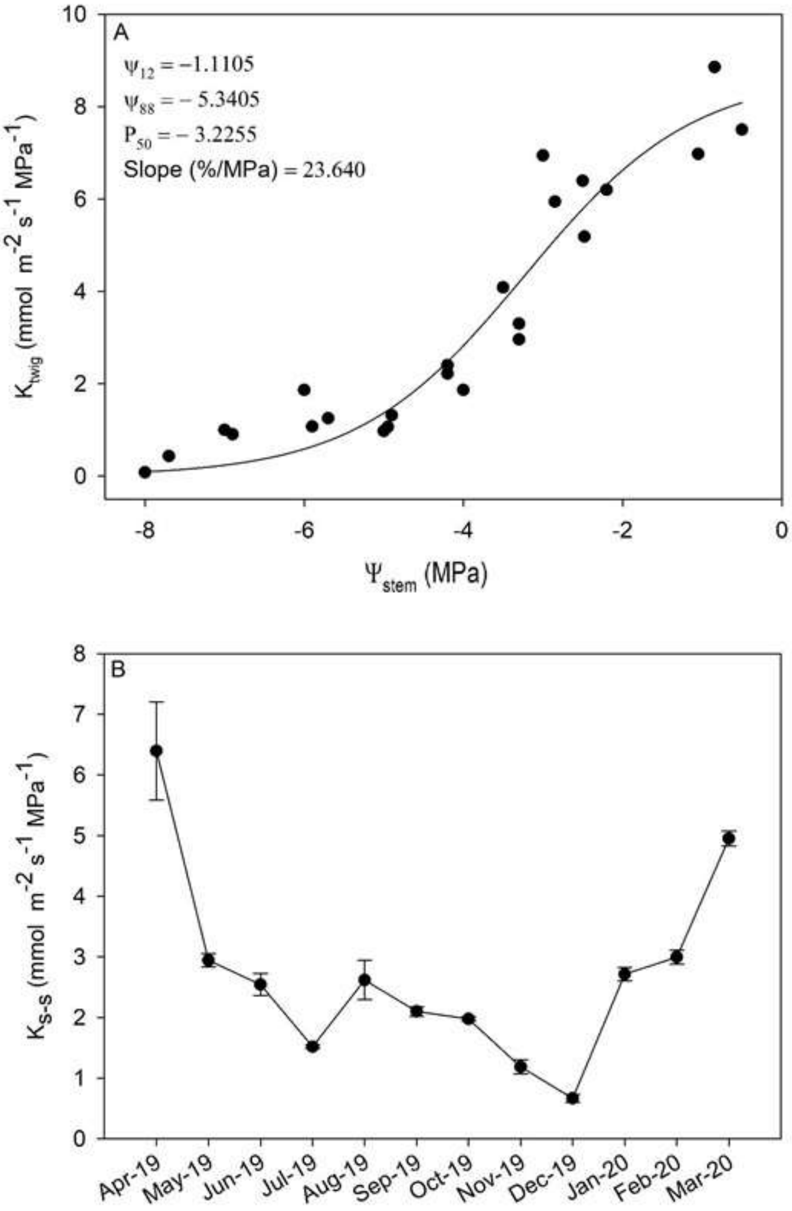

Figure 4: Hydarulic conductance as a function of time and water potential.

Twig hydraulic conductance (K_{twig}; A) as a function of stem water potential (Ψ_S) along the experiment. Plant hydraulic conductance ($K_{s\text{-}s}$; B) along the experiment. Data are means ± SE, n=4.

Discussion

Our results demonstrate olive trees' ability to sustain Ψ_{stem} of -8 MPa while maintaining substantial gas exchange throughout the dry summer and recovering full functionality without sacrificing leaves after the winter rains. The -8 MPa that we measured was similar to record lows Ψ_{stem} for olive trees under extreme drought (Angelopoulos *et al.*, 1996; Ennajeh *et al.*, 2008; Moriana *et al.*, 2003a) and is a testament to olives' remarkable drought tolerance. The fact that -8 MPa was never surpassed, not even during the seven months drought the trees experienced in the current experiment, might suggest that -8 MPa is the limit and that olives won't be able to recover lower Ψ_{stem}.

Osmotic adjustment

The results join a large number of studies that showed olive trees' ability to accumulate high concentrations of osmolytes in response to dehydration (Dichio *et al.*, 1996; Dichio *et al.*, 2006; Ennajeh *et al.*, 2008; Sofo *et al.*, 2008). The similar dynamics of Ψ_{stem} and π_{100} along the season imply that π_{100} is mostly affected by the plant water status rather than phenological effects. The only exception is the measured increase in π_{100} upon leaf maturation, which is long known for other species like Soybeans *(Glycine max)*, red and white oak (*Quercus Rubra, Quercus Alba*), and Carya (*Carya tomentosa*) (Zur *et al.*, 1981; Parker *et al.*, 1982). While we still don't know which osmolytes drive the changes during leaf maturation, (Fernandez-Escobar *et al.*, 1999) showed that Calcium (a major osmolyte in olive leaves; (Dichio *et al.*, 2009)) more than doubled its concentration during the first year of olive leaves maturation. This higher π of mature leaves implies that they are more resilient to water shortage and could explain why in the bench dehydrated shoots, the minimal g_s of 3 months old leaves was lower than the 15 months old leaves.

The daily adjustment of π between predawn and the afternoon highlights olive trees' ability to regulate their turgor in respect to the daily fluctuation in water potential. Our finding that the active adjustment account for nearly half of the total daily change in osmotic concentration is similar to the 42% measured by (Dichio *et al.*, 2006) in potted olives. A large daily osmotic adjustment was also measured in other species ((Bowman and Roberts, 1985; Gersony *et al.*, 2020), implying that the phenomenon is probably common among taxa. It seems reasonable that most of the daily-accumulated osmolytes are photosynthetic products that build up during the day and are exported through the phloem at night (Quick *et al.*, 1992; Gersony *et al.*, 2020). In support, the major osmolytes found in olives are Glucose and Mannitol (Cataldi *et al.*, 2000; Dichio *et al.*, 2009). Even at the end of the summer, under extreme water stress that led to minimal assimilation, there was a daily accumulation of osmolytes, probably thanks to reduced phloem export from the leaves under water stress (Woodruff, 2014).

Hydraulic conductance

Under stress, the reduction in K_{twig} was much more prominent than $K_{s\text{-}s}$, pointing that the hydraulic vulnerability segmentation hypothesis (Tyree and Ewers, 1991) is valid in olives. In support, other studies have also found that olive leaves are more vulnerable as compared with their stems (Torres-Ruiz *et al.*, 2015; Rodriguez-Dominguez *et al.*, 2018). Both K_{twig} and $K_{s\text{-}s}$ measurements imply that by the end of the summer, under -8 MPa, the plants have lost most of their hydraulic conductance, probably as a result of xylem cavitation. It could be argued that our measurements are inaccurate due the importance of outer-xylary regulation of conductance or the effect of compartmentalized capacitance that could effect the rehydration kinetics measurements (Blackman and Brodribb, 2011). However, even more direct methods to quantify xylem cavitation in olives showed that 50% cavitation (P_{50}) in stems or leaves was between -4.5 and -7 MPa (Ennajeh *et al.*, 2008; Torres-Ruiz *et al.*, 2014; Torres-Ruiz *et al.*, 2017; Rodriguez-Dominguez *et al.*, 2018). The fact that the minimal $K_{s\text{-}s}$ values were measured in November and December, when Ψ_{stem} were not at their minimum, is probably due to the low temperatures in these months that further reduced the plant hydraulic conductance thanks to their effect on viscosity and aquaporin expression (Murai-Hatano *et al.*, 2008). The recovery of $K_{s\text{-}s}$ in the spring was probably due to the differentiation of new xylem vessels that could have replaced the conductance that was lost at peak summer (Améglio *et al.*, 2002; Hammond *et al.*, 2019). These findings suggest that olives can tolerate and recover a high degree of xylem embolism, as was previously shown for beech, poplars and pines (Barigah *et al.*, 2013; Hammond *et al.*, 2019).

Stomatal conductance in the face of hydraulic failure

An interesting finding was that the trees maintained substantial gas exchange despite the low $\Psi_{stem.}$ As observed by many others for nearly every studied tree species, most stomatal closure occurred above -4 MPa (Figure 5;

Summarized by (Martin-StPaul *et al.,* 2017). However, the idea that from that point onward, the plants will minimize their transpiration (Blackman *et al.*, 2016) is not supported by the current findings. Also, the notion that plants regulated their g_s to a minimum in order to avoid Ψ_{stem} that might lead to xylem cavitation (Sperry *et al.,* 1988) is called into question. The seasonal lowest g_s was 23 mmol m^{-2} s^{-1}, several folds higher than the minimal g_s that was measured in the lab (3-5 mmol m^{-2} s^{-1}) or the minimal g_s reported for other species (1-10 mmol m^{-2} s^{-1}; (Duursma *et al.,* 2019). While the measurement of leaf vapor conductivity (termed in this paper stomatal conductance- g_s) includes both the stomata pores and the cuticular conductance, it is unlikely that cuticular conductance had a substantial contribution to the measured g_s.

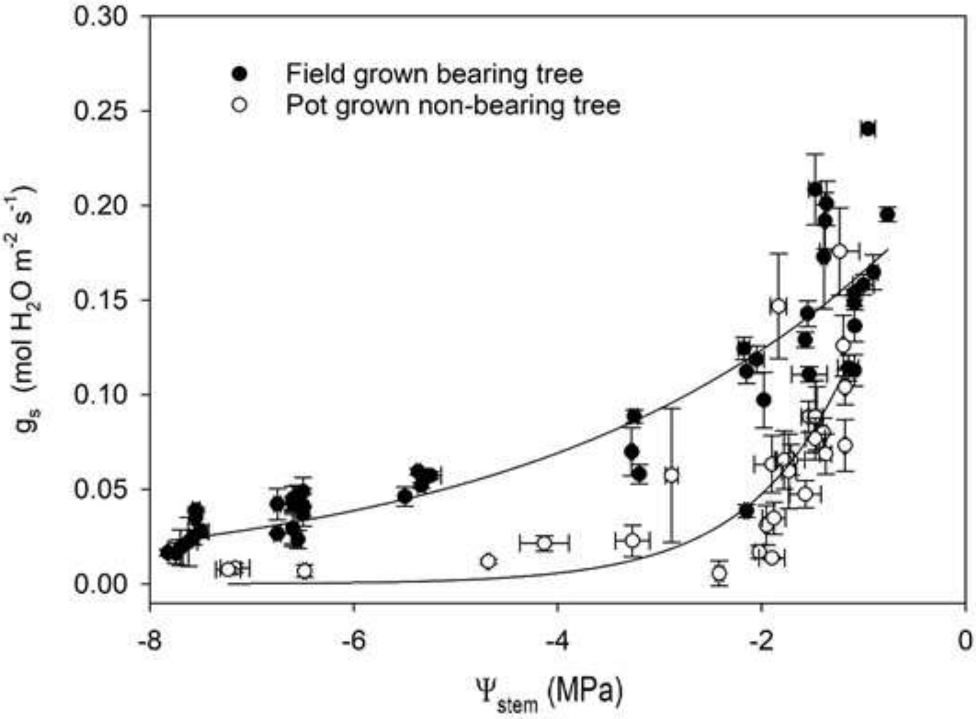

Figure 5: Comparison of stomatal closure in bearing and non-bearing olive trees.
Stomatal conductance (g_s) as a function of the stem water potential (Ψ_S) compared for potted young plants (CV. Souri) and mature bearing field grown trees (CV. Arbequina). The gs and Ψ_S were measured following the same protocols of the current paper during 34 days of dehydration. Complete experimental description is available in Barzilai *et al.* (2021). Data are means ± SE. For the field grown tree n=4 and for the potted tree n=6.

It is difficult to say if the relatively high g_s values that were measured under -8 MPa were kept thanks to turgor maintenance or despite negative turgor. Simulations of the leaf turgor using the apoplastic fractions that were previously measured for olives (from 10% in (Dichio *et al.,* 1996) to 40% in (Arias *et al.,* 2015)) do not clarify exactly if and when the trees lost turgor (Figure 6). However, it is more than likely that these relatively high g_s were maintained despite substantial cavitation (discussed above).

In agreement with our results, several other studies have also found that plants under extreme drought maintained g_s far above the minimal values. For example, (Odening *et al.,* 1979; Odening *et al.*, 1974) found stomatal opening and positive assimilation for *L. tridentata* at -8 MPa. Furthermore, (Hoffmann *et al.,* 2011), who investigated forests under extreme drought conditions in southeastern USA, found that despite extremely low predawn water potentials (<-4MPa) that led to substantial wilting and leaf desiccation, most plants exhibited g_s that was higher than 10 mmol m^{-2} s^{-1}. Also, in other olives experiments under low Ψ_{stem} (<-4 MPa), significant stomatal conductance was measured (0.25 < g_s <0.1 mol m^{-2} s^{-1}; (Moriana *et al.,* 2003a; Naor *et al.,* 2013; Perez-Martin *et al.,* 2014; Erel *et al.,* 2014; Torres-Ruiz *et al.,* 2015)). These findings remind us that the hydraulic signal is not the sole regulator of the stomata.

The high temperatures of the Mediterranean summer could increase minimal g_s by their effect on the cuticule integrity (Schuster *et al.*, 2016) or the leaf latent cooling requierments (Marchine *et al.*, 2021). Cuticular damage typically occurs at >40 °C (Schuster *et al.*, 2016; Billon *et al.*, 2020), while the maximal leaf temperature during measurement days (measured by the licor6400; File S1) was always lower than 38°C. However, we didn't measure on the hottest days of summer, and it is probable that leaf temperatures crossed the 40°C at some point of the season (see Fig S1), possibly increasing the cuticle permeability. Another possibility is that the hot summer temperatures (>30 °C in the measuring days of August, September and October) forced the trees to relatively high minimal g_s to maintain latent leaf cooling despite the cavitation danger.

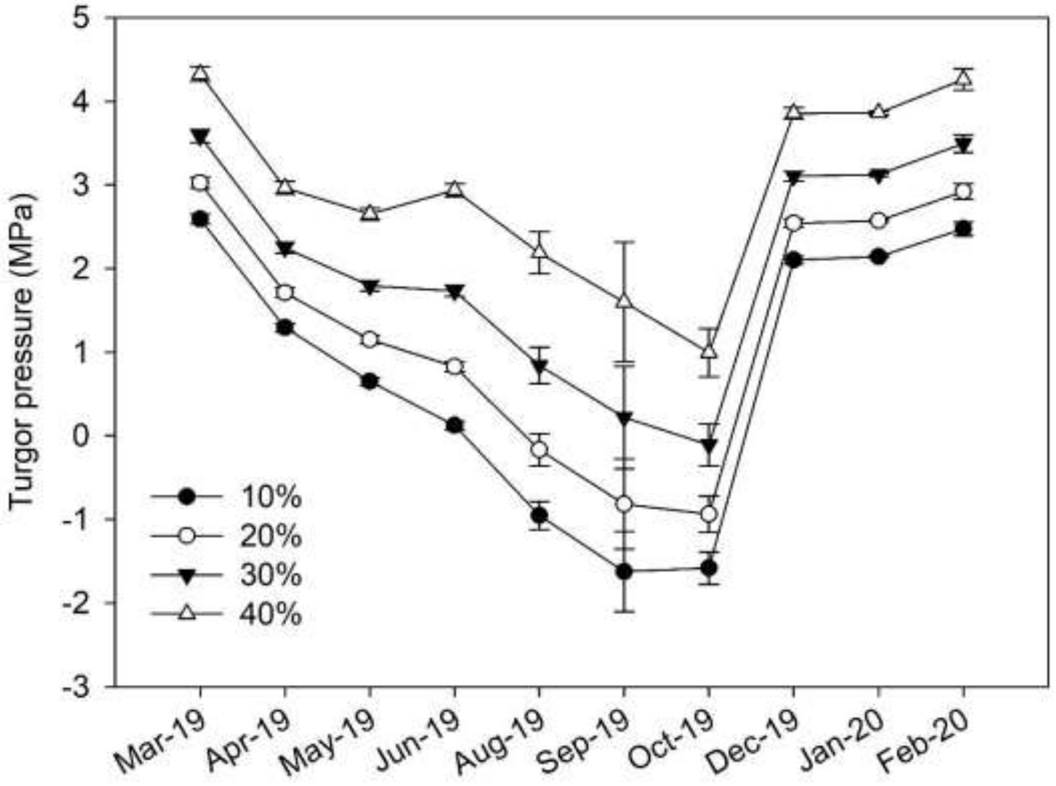

Figure 6: Turgor pressure simulations for four different apoplast fraction along the experiment.
Data are means ± SE, n=4.

In olives, perhaps more than in any other crop, fruit-bearing was shown to regulate stomatal conductance. (Bustan *et al.*, 2016) used large (2.5 m^3) lysimeters to show that fruit removal reduced transpiration by 30%. Additionally, stomatal response to water potential was shown to depend on the relative fruit load, where high fruit load led to substantial stomatal conductance even under very low Ψ_{stem} (Trentacoste *et al.*, 2011; Ben-Gal *et al.*, 2011; Naor *et al.*, 2013). In the only other study where field-grown olives were dried to -8MPa (Moriana *et al.*, 2003b), they exhibited g_s higher than 25 mmol m^{-2} s^{-1} in the 'on' year, but much lower g_s and higher Ψ_{stem} in the 'off' year. When reviewing the existing literature on olives water relations, it becomes apparent that complete stomatal closure under low Ψ_{stem} occurred only in non-bearing trees (Angelopoulos *et al.*, 1996; Sofo *et al.*, 2004; Perez-Martin *et al.*, 2014). Studies on fruit-bearing olive trees rarely measured stomatal conductance lower than 30 mmol m^{-2} s^{-1}. To emphasize the difference between dehydration of potted young trees and mature bearing trees, Figure 5 combines data of g_s in respect to Ψ_{stem} from the current study with a dataset that we recently collected for potted (10L) non-bearing olives (CV. Souri; Barzilai *et al.*, (2021)). The figure portrays the "unexpected" behavior of mature-bearing trees compared with the minimal stomatal conductance (<10 mmol m^{-2} s^{-1}) exhibited by the young potted trees from -4 MPa downward (Figure 5). One clear difference between potted and field trees is the root volume that when combined with low Ψ_{stem} enables sufficient suction to utilize deep water pools. Alternatively, these findings imply that gas exchange regulation is not strictly hydraulic and that other factors, such as temperatures and fruit load, should be considered when modeling water loss under extreme drought.

In conclusion, olive trees can sustain positive assimilation under the long and dry Israeli summer even when driven to a very low Ψ_{stem}, partially thanks to their ability to accumulate a large concentration of osmolytes. The trees lost most of their stomatal and hydraulic conductance during the dry period, but these were fully recovered after the winter rains. It seems that olive trees maintain their g_s above the minimal values even when surpassing the threshold of xylem cavitation.

Supplemental data

File S1. The entire raw data and statistical analysis of the data presented in figures 1,2, and 3.
Figure S1. Precipitation and Penman values during the experiment
Figure S2. Daily maximun and minimum temperature values during the experiment.
Figure S3. Sub cutictular carbon concentration (Ci) during the experiment.
Figure S4. The hydraulic conductivity between the soil and the stem (Ks-s) as a function of stem water potential (Ψ_{stem}) along the experiment

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