High sensitivity of hop plants (*Humulus lupulus* L.) to limited soil water availability: the role of stomata regulation and xylem vulnerability to embolism

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Abstract

Drought poses a serious threat to the productivity of hop, an important perennial crop. However, the precise physiological mechanisms that make it highly susceptible to drought are not yet fully understood. In this study, we investigated stomatal regulation and xylem vulnerability to embolism, which are important traits closely associated with plant drought resistance. In a glasshouse cultivation experiment, we monitored changes in leaf water potential, stem elongation rates, and leaf gas exchange, including net photosynthetic rates, stomatal conductance, and intrinsic water use efficiency, on relatively young hop plants (traditional Saaz - Osvald's clone 31) exposed to declining soil water availability. The transpiration rate and stem elongation of plants decreased significantly with a small decline in substrate water potential (Ψ_{SUB}), indicating a highly sensitive stomata response during early phases of soil dehydration. The stem elongation was completely halted, and the transpiration rate dropped to less than 50% of its maximum at Ψ_{SUB} levels below -0.8 MPa. In well-watered hop plants, xylem in stems operates near the initial point of embolization and is highly vulnerable to embolism, with a water potential corresponding to a 50% loss of xylem conductivity at -1.6 MPa. The sensitive stomatal response to declining Ψ_{SUB} likely helps to mitigate the risk of hydraulic failure, albeit at the cost of impaired growth. Scheduled irrigation, particularly during the sensitive stem elongation stage, may be a promising approach to mitigate the detrimental effects of reduced soil water availability on hop growth and yield while also conserving water resources.

Keywords Leaf water potential · Xylem transport · Vulnerability curve · Irrigation management · Drought · Water use efficiency

Introduction

Changes in precipitation patterns in recent years have significantly impacted crop production, particularly due to the overall reduction and irregular seasonal distribution of rainfall (Hlavinka et al. 2009; Hari et al. 2020). Compared to most other crops, common hop (*Humulus lupulus* L.) is highly sensitive to limited soil water availability, which affects its growth, yield, and the quality of hop cones. The sensitivity of hops to water scarcity has been shown by a significant negative correlation of yield with summer precipitation monitored over the past fifty years (Mozny et al. 2009). Therefore, maintaining stable hop yields and high-quality hop cones will be more challenging due to the rapid pace of ongoing climatic changes (Potopová et al. 2021; Mozny et al. 2023).

Hop is a perennial plant that produces climbing annual shoots. These fast-growing shoots can grow more than ten meters long and are supported by thin stems that are only up to ten millimeters in diameter (Rybacek et al., 2012). Hop plants are widely cultivated in many countries, including the Czech Republic, Germany, and throughout Europe, with a total of 5, 21, and 29 thousand hectares, respectively (Hop Harvest Report, 2022). Despite the agricultural significance of this crop, there is a surprising lack of information regarding the physiological responses of hop plants to drought (Hejnák et al. 2015; Kolenc et al. 2016; Jupa et al. 2016a).

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This lack of understanding hinders the development of efficient cultivation techniques and the breeding of cultivars with stable yields under fluctuating water availability. Irrigation systems are commonly used to stabilize hop yields (Mozny et al. 2009). However, the limited availability of water resources is increasingly restricting the wider use of irrigation, which is driving the need for more sophisticated methods of irrigation based on plant response to water availability. Therefore, growers and breeders urgently require detailed information about the water relations of hop plants, particularly their response to water availability, to stabilize hop yields under future climatic scenarios.

The change in stomatal conductance is a crucial mechanism for regulating water usage in plants when water availability fluctuates (Buckley 2019). Some species with sensitive stomatal regulation begin closing their stomata at relatively high soil water availability to minimise water loss and maintain good hydration during prolonged soil drought. However, early stomatal closure can also negatively impact plant growth by reducing carbon gain (Potkay et al. 2021). The growth and development of fast-growing crops, such as hop plants, may be significantly limited by the negative impacts of sensitive stomatal response to low water availability. In contrast, less sensitive stomatal response allows plants to keep stomata widely open even under more significant reductions in water availability (Hochberg et al. 2018). The plant's ability to maximize growth and carbon uptake is enhanced by its lower stomatal sensitivity. However, this also increases the risk of accelerated tissue dehydration under drought conditions, which can lead to the failure of long-distance xylem transport (Martinez-Vilalta and Garcia-Forner 2017).

Maintaining good tissue hydration is crucial for plant growth. Cell turgor, specifically increased water flux into the cell along the osmotic gradient, is the main force behind elongation growth (Ali et al. 2023). Therefore, reduced water supply can significantly affect elongation growth and indicate plant sensitivity to water scarcity in soil. (Nonami 1998).

Prolonged exposure to water scarcity can lead to a decrease in xylem water potential if tissue water reserves are rapidly depleted and not replenished by uptake from dry soil, and can result in the extensive formation of embolism. The blockage of more xylem conduits by air increases the total hydraulic resistance of the xylem transport pathway, resulting in a decreased flow rate and limited water supply to distal organs (Sperry et al. 2008). Under high transpiration rates, limited water delivery may lead to decreased water content in tissues and decreased shoot water potential (Hacke and Sauter 1995). Catastrophic failure of xylem transport by extreme or chronic drought stress frequently results in dieback of the organ or whole individual (Choat et

al. 2012, 2018). Therefore, angiosperm woody species have several protective mechanisms to prevent failure of xylem transport under drought stress. Conservative stomatal regulation (Nardini and Saleo 2000; Brodribb and Holbrook 2003), high transport capacity and redundancy of xylem conduits (Tyree and Zimmermann 2002), specific structural traits of conductive elements limiting embolism spread (e.g., isolated conduits, thick pit membranes; Wheeler et al. 2005; Li et al. 2016), high organ water storage capacity (Scholz et al. 2011; Jupa et al. 2016b) or efficient insulation properties of dermal tissues (Rosell 2016; Rosell et al. 2017) are examples of mechanisms involved in preventing hydraulic failure. These mechanisms have been previously observed in woody species, but they are not always applicable in lianas. In lianas with long and thin stems and a limited number of wide and conductive elements, hydraulic failure of only a few conduits due to embolism can significantly reduce stem transport capacity (Jacobsen et al. 2012; Jupa et al. 2013). Furthermore, the limited storage capacity of stem tissues (Köcher et al. 2013; Rosell et al. 2017) may restrict the ability to mitigate decreases in water potential in the xylem over both short and long-term periods. The apparent lack of functional significance of many protective mechanisms found in trees suggests that hops and other liana species may primarily rely on sensitive stomatal regulation to prevent extensive embolism formation and safeguard the vital function of the xylem during drought stress.

Our previous studies have shown that hop plants make sensitive adjustments to the structure of the xylem transport pathway during limited water availability (Jupa et al. 2016a). Additionally, long-distance regulatory signalling between roots and shoots has been examined under declining water availability (Korovetska et al. 2014). Furthermore, significant differences in the response of transpiration to drought have been observed among hop cultivars (Gloser et al. 2013; Korovetska et al. 2014). However, information on the dynamics of stomatal regulation under decreasing water availability and its association with the vulnerability of xylem conduits to embolism and plant growth is currently insufficient or missing. However, these mechanisms could help elucidate the high sensitivity of hops to drought.

This paper examines the relationships between stomatal regulation, xylem vulnerability to embolism, and stem elongation of young hop plants under decreasing soil water availability. The experiments were conducted on relatively young plants that reach high elongation rates because they may show the most sensitive responses to water shortage. Furthermore, it is necessary to comprehend the effects of limited water supply on plants during early vegetative growth. This is particularly important as hop plants in their early developmental stage may be the most susceptible to the adverse effects of water scarcity during their vegetation

cycle. Restricted vegetative growth during the initial phase of shoot ontogeny can significantly reduce the development of flowers and the final yield of hop cones (Rybacek 2012). We hypothesised that hops exploit sensitive stomatal regulation in response to decreasing substrate water availability, which may help this species to avoid a drop in water potential and excessive embolism formation under reducing water availability. However, sensitive stomata closing in the early stages of declining water availability would significantly limit carbon gain and plant growth.

Materials and methods

Plant material and experimental setup

First-year cuttings of virus-free hop plants (traditional Saaz Osvald's clone 31) were cultivated in a glasshouse for six weeks starting from the end of April. The Saaz hops cultivar is the most commonly cultivated variety in the Czech Republic and has been previously identified as sensitive to drought (Korovetska et al. 2014). Forty-four plants were individually planted in 10 L containers filled with a peat-based mixed substrate and attached to bamboo poles for shoot support. The plants were watered daily with tap water until the substrate reached full water capacity. Additionally, they received 100 ml of fertiliser solution (1.5 g l^{-1}) every week (Ferty 3; Planta Düngemittel, Regenstauf, Germany). The solution contained the following concentrations of major nutrients (in mol m^{-3}): 4.82 NO_3^-, 11.24 NH_4^+, 1.06 mM P_2O_5, 2.39 K_2O, 0.74 MgO, and 0.02 Fe. Throughout the experimental period, the maximum daily temperatures were approximately 27 °C, and the maximum water vapor pressure deficit (VPD) was approximately 2.3 kPa. Figure 3A (day 5 of drying) presents the representative diurnal patterns of microclimatic conditions in the greenhouse. The plants were grown under natural daylight without the use of supplemental lights, for approximately 16 h from 5 a.m. to 9 p.m. Once the plants reached a height of approximately 3 m (BBCH 18–19; Rossbauer et al. 1995), half of them (twenty -two plants) were randomly selected among all plants in the glasshouse to minimize the effects of any environmental heterogeneity. These plants were subjected to the Water Limited treatment (WL) by ceasing watering at the start of the experimental period. The second group of plants, which received the Well-Watered treatment (WW), were watered daily with tap water up to the full field capacity of the substrate, as previously determined according to Baize (1993). Cessation of watering in WL plants resulted in an exponential decrease in the substrate water content in time (WC$_{SUB;}$ see Supplemental information (SI) 1). We started measurements of the physiological parameters (see below) four days after watering cessation and concluded eight days later. According to our previous experience, the physiological response of hop plants occurred after a significant decline in WC$_{SUB}$. At the start of the physiological measurements (day 4), the mean WC$_{SUB}$ in the WL treatment decreased significantly below the WW treatment and ranged between 0.8 and 0.9 g g^{-1}, which is 40–50% of that in the WW treatment (corresponding substrate water potential Ψ_{SUB} = -0.3 to -0.5 MPa; SI 1). At the end of the physiological measurements on day 8, WC$_{SUB}$ in the WL treatment decreased to 0.3 g g^{-1}, which is approximately 20% of the WW treatment (Ψ_{SUB} = -1.9 MPa; SI 1). To ensure complete trends in physiological responses, we performed measurements of physiological parameters on a set of plants for four consecutive days of drying, as the plants differed in leaf area and their substrate was desiccating at different rates. Plants from each treatment were examined in random order every day. To minimize the effects of diurnal patterns, we took measurements of randomly selected plants from both WW and WL treatments alternately. We continued taking measurements until the stomata of WL plants remained closed during the day and transpiration rates (TR) stabilized at a minimum. Each day when physiological measurements were performed, WC$_{SUB}$ was monitored in each container later in the afternoon using an ML2x ThetaProbe sensor connected to the HH2 data logger (Delta-T Devices Ltd., UK). The mean value of three measurements per container was recorded. WC$_{SUB}$ values were then converted to Ψ_{SUB} using a calibration curve constructed with the WP4C Dewpoint Potential Meter (Decagon devices, Pullman, USA; SI 2).

Leaf water potential, transpiration and elongation rates

Leaf water potential (Ψ_L) was measured on leaf blade samples using the psychrometric technique using a micro-voltmeter HR-33T (Wescor Inc., South Logan, UT, USA) connected to the C-52 sample chamber. In previous experiments, measurements of Ψ_L using a Scholander pressure chamber method produced inconsistent and highly variable results of balance pressure, so the pressure chamber method was unsuitable for this species. Two leaves in the central part of the stem were chosen for Ψ_L measurements. Two 10 mm diameter discs were taken from each leaf blade and combined as a sample. The sample was then placed in a chamber and allowed to equilibrate for 25 min under thermally stable conditions. Ψ_L was determined by converting microvolt readings to Ψ_L using a calibration curve. When examining the relationship with gas exchange parameters, Ψ_L was determined within 30 min of the gas exchange measurement.

Transpiration rates (TR) were determined using the gravimetric method. Six intact plants from each treatment were weighed every two hours. Containers were covered with plastic bags to prevent direct evaporation of water from the soil. Leaf area was determined for each plant based on the known ratio between leaf area and leaf dry mass. The ratio was calculated based on the leaf area and dry mass of all leaves harvested from three WW plants at the end of the experiment. Leaf area was determined using image analysis software ImageJ to analyse scanned images of individual leaves (NIMH, Bethesda, MD, USA).

Stem elongation rates (ER) were measured on the terminal part of the stem, approximately 30 cm from the apex, of six selected plants in each treatment during the experimental period. The distance from the apex to the nearest developed node was recorded once daily at approximately 6 p.m. for three consecutive days, providing two intervals for the calculation of ER for each plant. The mean ER value was calculated from data from both intervals for each plant. The Mean ER data were then used to construct the relationship between ER and corresponding Ψ_{SUB}.

Transpiration rates were measured for each plant between days 4 and 8, from 10 a.m. to 2 p.m. To gain a better understanding of how hop plants respond to reduced water availability, we examined the diurnal changes in TR and Ψ_L of WW and WL plants when WC_{SUB} decreased below 50% of full saturation on day 5 of the drying period. On that day, TR was determined using one hour weighing intervals to better understand the time course. Data for ER calculation were collected on days 4, 5, and 6 of the experiment.

Minimum stem water potential and vulnerability of xylem to embolism

Samples for measurements of minimum stem water potential (Ψ_{MS}) and xylem hydraulic conductivity were collected three days after the drying experiment ended from a subset of WW plants that were cultivated in parallel with the rest of the experimental plants. The Ψ_{MS} was measured on bagged leaves in the middle of the stem segment during midday using the psychrometric technique described for Ψ_L above. The leaves were bagged one hour before measurement.

Segments for hydraulic measurements were sampled in the central part of the stem starting approximately 1 m from the shoot tip and had a total length of about 1.5 m. All but one leaf from the stem were removed, stems were cut under water and silicone tubes filled with distilled water were connected to both stem ends to avoid stem dehydration. Leaf petiole cuts were sealed with glue, and the last attached leaf was wrapped in a small plastic bag. The segments were transported horizontally to the laboratory in a large plastic bag with moist paper towels for measurements.

Xylem vulnerability to embolism was determined in the laboratory using the bench dehydration method described by Sperry et al. (1988). Briefly, the excised stem segment with removed silicone tubes from both ends was left to continuously dehydrate at laboratory temperature for varying periods. Segments with zero dehydration time were considered to contain native embolism. Subsequently, the stem water potential (Ψ_S) was measured on two leaf discs that were sampled from the bagged leaf using the psychrometric technique described above. The stem segment was then shortened several times under water from each side (for approximately 20 cm) with a razor blade close to the nodes to prevent solution leaks through the hollow stem internodes. The segment was always longer than 1 m, which is longer than the longest vessels found in this species (< 75 cm; Gloser et al. 2011; Jupa et al. 2013). Following stem dehydration, the native hydraulic conductivity (K_h; m^4 MPa^{-1} s^{-1}) of the stem xylem was determined using a tubing apparatus directing 10 mM KCl solution through the stem segment to a container placed on a precise analytical balance (Excellence XS-205, Mettler-Toledo, Germany). The flow rates under a pressure head of approximately 8 kPa were continuously recorded in a computer with custom data acquisition software. The K_h value was calculated using the acquired steady-state flow rate (f; m^3 s^{-1}) and pressure gradient (ΔP; MPa m^{-1}) values, following the equation:

$$K_h = \frac{f}{\Delta P}$$

The stem segments were then perfused with a KCl solution at a pressure of 150 kPa for 15 min to fully remove any embolism. Maximum hydraulic conductivity (K_{max}) was measured in the same manner as K_h, as described above. The pressure and duration of the perfusion were determined to be sufficient for complete embolism removal, as previously measured (SI 3). PLC (%), the percentage loss of hydraulic conductivity due to embolism, was calculated for each stem segment using the following equation:

$$PLC = 100(K_{max} - K_h)/K_{max}$$

Values of PLC from all samples were plotted against Ψ_S, and the data were fitted with a sigmoid function following Pammenter and Vander Willigen (1998):

$$PLC = \frac{100}{1 + \exp a(\Psi_S - \Psi_{50})}$$

where a is a constant related to the curve slope, and Ψ_{50} corresponds to the Ψ_S at 50% loss of hydraulic conductivity.

In addition, other parameters characterising xylem vulnerability to embolism were calculated according to the following equations (see Domec and Gartner 2001 for details) - Ψ_{12} (i.e., Ψ_S at 12% loss of hydraulic conductivity - corresponding to the initial point of embolization - i.e. air entry point), Ψ_{88} (i.e., Ψ_S at 88% loss of hydraulic conductivity - corresponding to the point of irreversible embolization for angiosperm species; Choat et al. 2012)d (i.e., the slope of the steep increase course of the vulnerability curve):

$$\Psi_{12} = \frac{2}{a} + \Psi_{50}$$

$$\Psi_{88} = -\frac{2}{a} + \Psi_{50}$$

$$S = 100a/4$$

Leaf gas exchange

Net photosynthetic CO_2 uptake rate (A; µmol m^{-2} s^{-1}) and stomatal conductance (g_S; mmol m^{-2} s^{-1}) were measured repeatedly on five randomly selected plants from each treatment between days 4 and 8 after watering cessation. Leaf gas exchange was measured on one fully developed leaf at mid-stem length of each plant using a CIRAS-2 portable infrared gas exchange system equipped with a temperature-controlled leaf cuvette (PLC3; PP-Systems, Amesbury, USA) with a light source. Measurements were performed at a leaf temperature of 25 ± 0.5 °C and a VPD of 1.1 ± 0.2 kPa. Photosynthetic photon flux density at the leaf surface was maintained at 900 ± 50 µmol m^{-2} s^{-1}, which ensured light saturation of photosynthesis in leaves of this species. The concentration of reference CO_2 entering the cuvette was 380 ppm and the concentration leaving in the cuvette was 40–60 ppm lower. Gas exchange measurements were performed from 10 a.m. to 3 p.m. The Ψ_L was determined by HR-33T shortly after each gas exchange measurement. Intrinsic water use efficiency (WUE; µmol CO_2 mmol^{-1} H_2O) was calculated as: WUE $= A/g_S$.

Statistical analysis

Statistica v. 12 (StatSoft Inc., Tulsa, OK, USA) was used for all statistical analyses. Simple linear and nonlinear regression analyses were applied to assess relationships between investigated parameters. All fitted lines shown in the graphs represent statistically significant relationships. All results were considered statistically significant at $P < 0.05$.
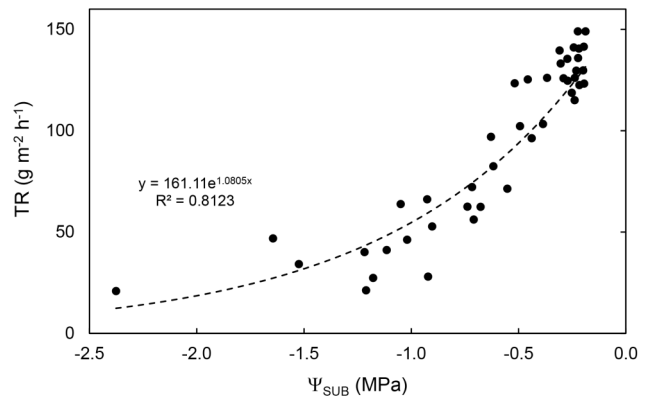
Fig. 1 The relationships between whole plant transpiration rate (TR) and substrate water potential (Ψ_{SUB}) of *H. lupulus* plants. Each point represents the measurement of one plant. Data were fitted using an exponential function
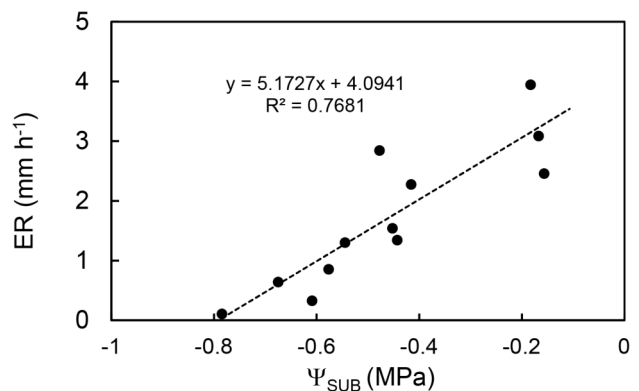
Fig. 2 The relationship between the stem elongation rate (ER) and substrate water potential (Ψ_{SUB}) of *H. lupulus* plants. Each point represents the mean response of one plant in two of the measured intervals. Data were fitted using a linear function

Results

Transpiration rates, leaf water potentials and elongation rates

In response to substrate desiccation, hop plants exponentially decreased TR along with the decline of Ψ_{SUB} (Fig. 1). We observed a steep decrease in TR in the early stages of substrate desiccation. Specifically, the initial decrease in Ψ_{SUB} to -1 MPa resulted in a reduction of TR of approximately 70%. The minimum TR started to level off after a decline of Ψ_{SUB} below -1.5 MPa, corresponding to a residual TR of about 20% of the maximum TR of fully watered plants (Fig. 1).

Like TR, the stem elongation rates were very sensitive to substrate water availability. The elongation rate (ER) decreased linearly along with decreasing Ψ_{SUB} (Fig. 2). The elongation stopped completely when the Ψ_{SUB} fell below -0.8 MPa (Fig. 2).

We also analyzed diurnal changes in transpiration rates and leaf water potentials around the middle of the drought period on day 5. During day 5, WW and WL plants were exposed to mean Ψ_{SUB} of about -0.18 and -1.10 MPa, respectively. Under these conditions, we observed remarkable differences between WW and WL treatments in the day courses of TR and Ψ_L. The transpiration rates of WW plants increased steeply during the morning hours and peaked at noon, although the VPD reached its maximum (>2 kPa) several hours later (around 4 p.m.; Fig. 3A-B). In contrast, the maximum daily TR in WL plants reached only 30% of the rate recorded for WW plants (Fig. 3B). At the beginning of the photoperiod, a much more negative Ψ_L was detected in WL compared to the WW treatment (-1.21 MPa for WL,

-0.47 MPa for WW plants), and this difference between treatments increased towards the midday minima (-2.45 and -1.07 MPa, Fig. 3C) due to a steeper decrease of Ψ_L in the WL treatment.

Leaf gas exchange

We analysed relationships among g_S, A, WUE and Ψ_{SUB} to understand better the impacts of substrate water availability on plant carbon gain. Maximum g_S and A measured in WW plants were around 350 mmol m^{-2} s^{-1} and 13 μmol m^{-2} s^{-1}, respectively. We found a hyperbolic relationship between A and g_S, showing a non-linear response of A to decreasing g_S (Fig. 4). Along with the initial decrease of g_S,

Fig. 3 (**A**) Typical time course of temperature (T), relative air humidity (RH) and vapour pressure deficit (VPD) during experimental measurements in the glasshouse. (**B**) diurnal changes of the transpiration rate (TR) and (**C**) leaf water potential (Ψ_L) of *H. lupulus* plants cultivated in two treatments with different substrate water availability: well-watered (WW, mean Ψ_{SUB} = -0.18 MPa) and water-limited (WL, mean Ψ_{SUB} = -1.1 MPa). The climatic and physiological data were obtained on day 5 of drying. The natural daylight period (no supplemental lights were used) was approx. 16 h - from 5 a.m. till 9 p.m. Data points and error bars represent mean and SE. Number of replicates was 6 and 3 for graph B and C, respectively
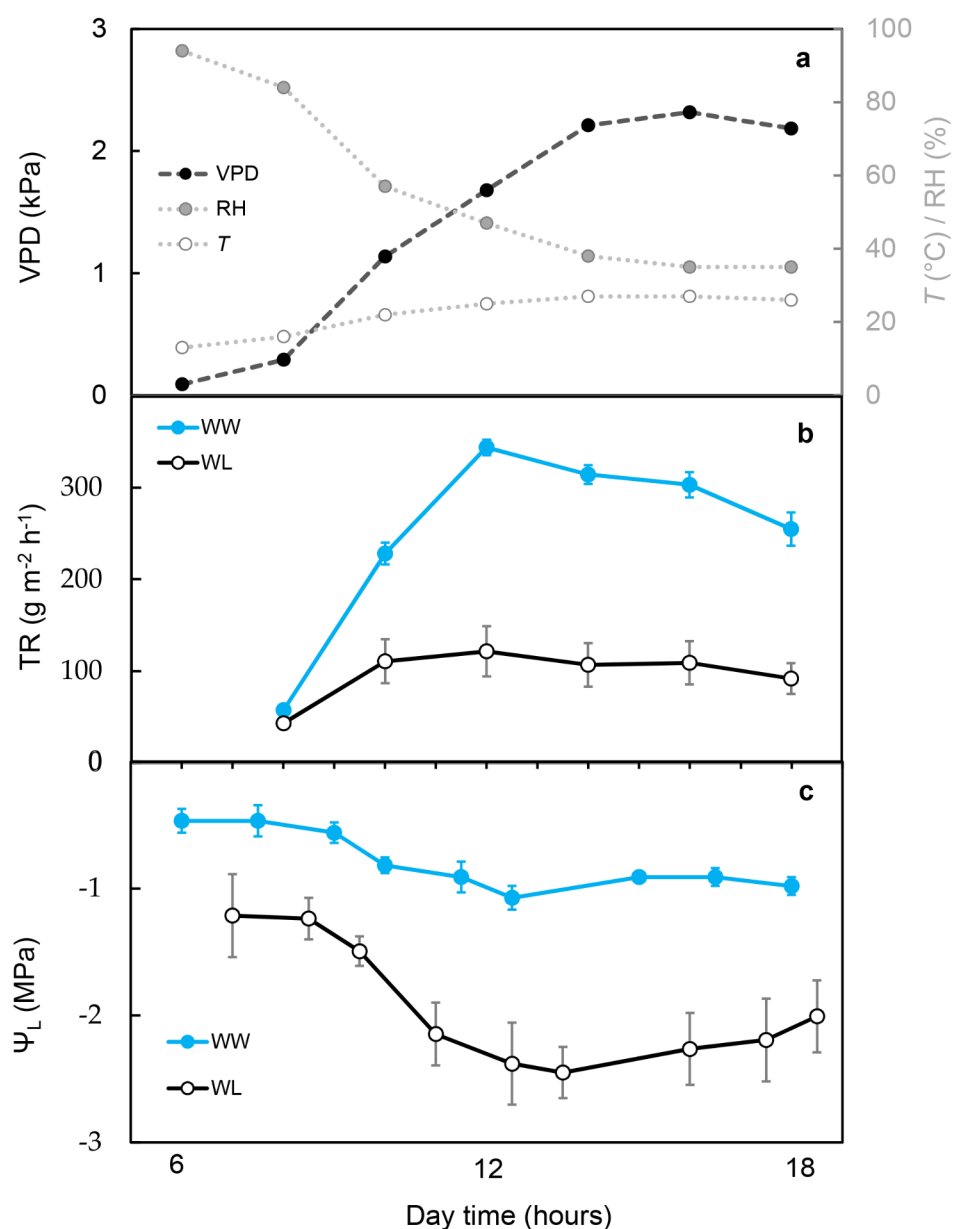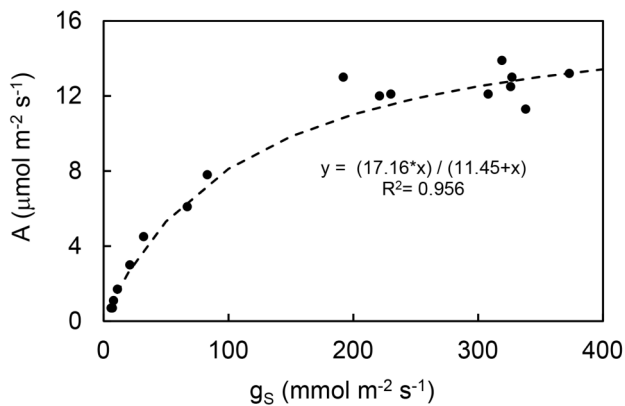
Fig. 4 The relationship of the net photosynthetic rate (*A*) and corresponding stomatal conductance (*g*$_S$) of *H. lupulus* leaves. The data were fitted using a hyperbolic function. Each point represents the independent measurement of one plant

hops maintained high and relatively stable photosynthetic rates. The decline of *A* became steeper when *g*$_S$ was reduced below 100 mmol m^{-2} s^{-1}. Besides, leaf gas exchange sensitively responded to the decreasing substrate water availability, as *g*$_S$, *A* and WUE were closely correlated with Ψ_{SUB} (Fig. 5A-C). The decline in Ψ_{SUB} from the recorded maximum (-0.2 MPa) below -1 MPa was associated with a prominent reduction of *g*$_S$ by approx. 60% (Fig. 5A). The *g*$_S$ was reduced to a minimum (i.e., $< 10\%$ of the maximum) when Ψ_{SUB} was lower than -1.5 MPa.

Although the rate of net photosynthesis reflected *g*$_S$, the decline of *A* in response to Ψ_{SUB} was not as steep as *g*$_S$ (Fig. 5B). Consistent with the steeper decline of *g*$_S$ than *A* with decreasing Ψ_{SUB}, we observed a steep linear increase in WUE with decreasing Ψ_{SUB} (Fig. 5C). The relationships for individual gas exchange parameters with Ψ_L were very similar to those with Ψ_{SUB} (Fig. 5D-F).

Vulnerability of the xylem to embolism

We found a typical sigmoid relationship between stem water potential and PLC with $\Psi_{50} = $ -1.62 MPa (Fig. 6). According to the other functional parameters obtained from the curve, intensive embolism formation starts at $\Psi_{12} = $ -0.97 MPa, which translates into a very steep increase of PLC (S = 77.30% MPa^{-1}) and reaches Ψ_{88} at -2.27 MPa. The Ψ_{MS} of WW hop plants was similar to Ψ_{12}, reaching -0.97 ± 0.17 MPa.

Discussion

In this study, we investigated the relationships between stomatal regulation, xylem vulnerability to embolism, and growth of young hop plants in response to decreasing soil water availability. We hypothesized that sensitive stomatal closing in response to decreasing soil water availability might help hops avoid a decrease in water potential, but early stomatal closure might simultaneously limit carbon gain and plant growth. Consistent with our hypothesis, hops sensitively decreased *g*$_S$ and TR in response to decreasing Ψ_{SUB}, accompanied by a steep reduction in stem growth. At the same time, we found that hop xylem operates at a water potential close to the air entry point, and that xylem transport pathways are highly vulnerable to embolism.

Similar to hops, sensitive stomatal regulation and high xylem susceptibility to embolism have also been reported in other climbing plants, such as ivy or several tropical liana species (Leuzinger et al. 2011; Chen et al. 2017). The sensitive early response to decreasing substrate water availability is likely mediated by long-distance signals from roots to leaves. However, the nature of the signal is still under debate. Based on our previous results, some anions (e.g. sulfate) or other messengers seem to be the initial factor responsible for the exceptional stomatal sensitivity in hops. At the same time, the contribution of increased long-distance ABA transport is rather marginal (Korovetska et al. 2014). Alternatively, hydraulic signals may also be responsible for early stomatal closing, but this signalling mechanism is currently even less understood and has not been studied in hops (Salleo et al. 2000; Domec et al. 2010).

The sensitive response of stomata to decreasing water availability had several physiological consequences for hop plants. Reduced water supply through the xylem and decrease of turgor were probably responsible for the rapid decrease of elongation growth in WL plants, which was already observed at moderate decreases in Ψ_{SUB} (Fig. 2). The mechanism responsible for changes in ER under drought can be better clarified using parameters obtained from pressure-volume curves (Koide et al. 1989). However, the use of the pressure chamber method for Ψ_L measurements on hop leaves gave inconsistent results, which prevented a reliable assessment of pressure-volume curves. Some alternative methods to obtain information on turgor loss point, bulk modulus of elasticity or osmotic potential at full turgor, optimized for hop plants, will be needed in future experiments.

Regardless of the mechanisms, the sensitive reduction of ER under moderately reduced water availability can cause a severe delay in hop development in the early phase of plant ontogeny. The delay in elongation growth and the time when the hop stem reaches the top of the support was previously shown to correlate with flower formation and total hop cone yield (Acosta-Rangel et al. 2021). Therefore, even a slight decrease in water availability during the early developmental stages corresponding to intensive vegetative growth will
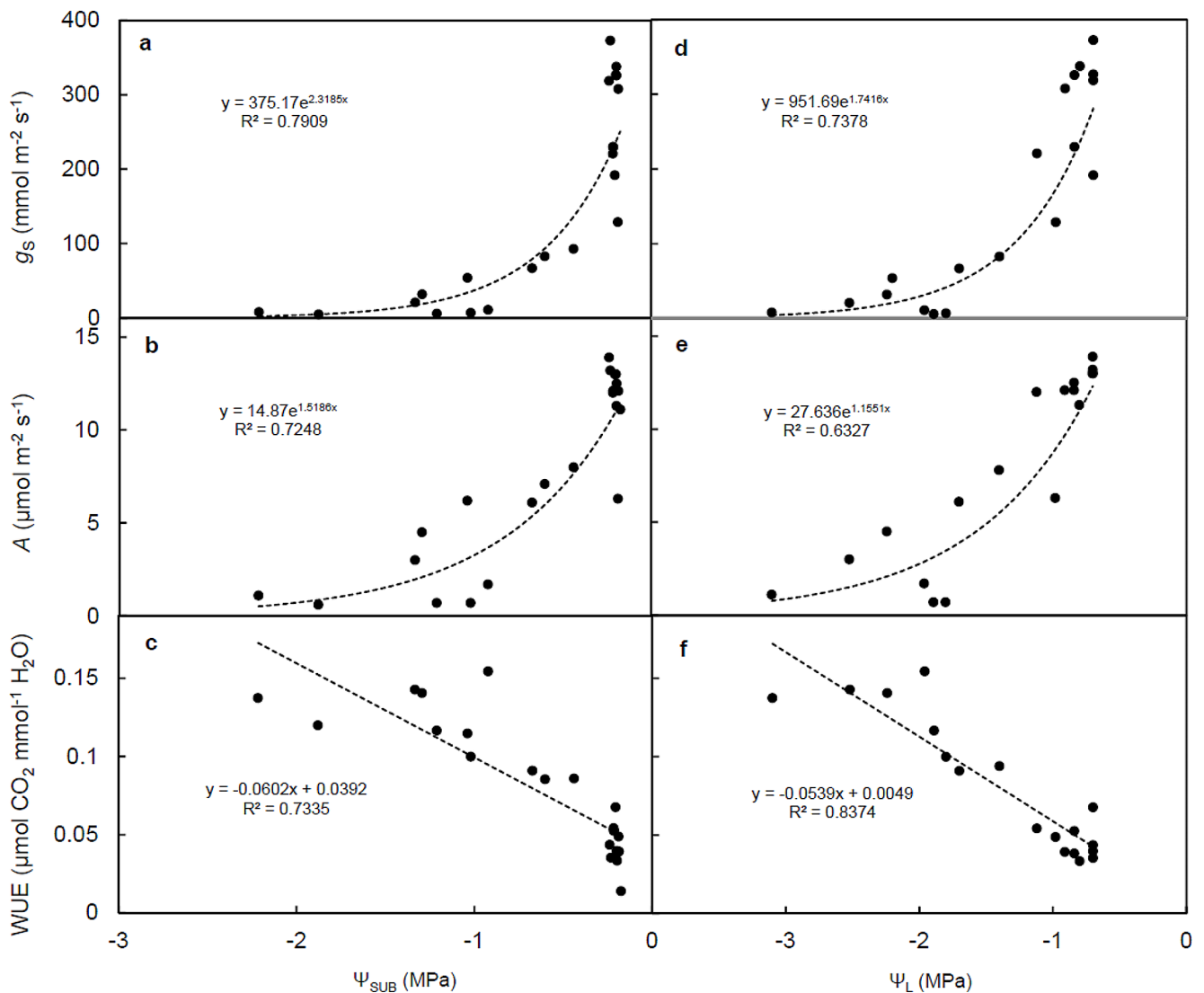
Fig. 5 (**A, D**) Stomatal conductance (g_S), (**B, E**) net photosynthetic rate (*A*) and (**C, F**) water use efficiency (WUE) of *H. lupulus* plants in relation to substrate water potential (Ψ_{SUB}; A-C) and to midday leaf water potential (Ψ_L; **D-F**). The data in graphs A, B, D and E were fitted using exponential functions, while those in graphs C and F were fitted using linear functions. Each point represents the independent measurement of one plant
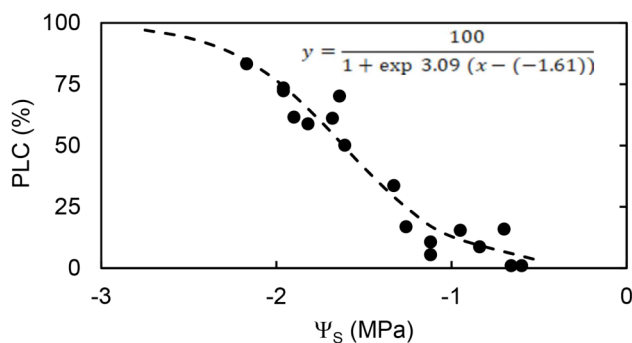
Fig. 6 The relationship between the percentage loss of hydraulic conductivity (PLC) and stem water potential (Ψ_S) of *H. lupulus* plants. The data were fitted using a sigmoid function. Each point represents the measurement of one stem sample per plant

most likely result in reduced productivity of hop plants in the current season.

Plants grow most intensively at night (Zweifel et al. 2021) and for that is sufficient cell turgor necessary. Therefore, pre-dawn water potential (Ψ_{PD}) is an important determinant of a plant's ability to grow under a given water availability. Although we did not measure Ψ_{PD} directly, our data on diurnal changes suggest that Ψ_{PD} remained lower in WL-treated plants than in WW plants (since day 5). Since VPD was close to 0 kPa and TR was negligible during the early morning hours (Fig. 3A and B), Ψ_{PD} should correspond relatively well to Ψ_L measured early in the morning (see Fig. 3C). The values of Ψ_{PD} roughly corresponded to Ψ_{SUB} as observed in other experiments with container-grown

plants (Ennajeh et al. [2008]). Our data on daily changes suggest that drought-exposed hop plants could not recover water potential sufficiently overnight to sustain growth. It is important to emphasize that Ψ_{PD} can differ significantly from Ψ_{SUB} in field-grown plants (e.g., Groenveld et al. [2023]). Its determination is therefore particularly important for field experiments.

Analysis of the gas exchange response revealed a remarkable increase in WUE with decreasing Ψ_{SUB} (Fig. [5]C). The increase of WUE with decreasing substrate water availability has been previously observed in several species (Flexas et al. [2002]; Edwards et al. [2011]; Qu et al. [2017]; Yang et al. [2021]). It suggests that plants can maintain carbon gain even with progressively reduced g_S. Reduced water loss after a decrease of g_S inevitably leads to reduced leaf CO_2 concentration. This effect was probably the main reason for the observed decline of A. In hop plants, however, was this reduction of A in response to declining g_S smaller than in another climber, *Vitis vinifera* (Flexas et al. [2002]). In this study, a 75% reduction in g_S resulted in a more than 50% reduction in A in *Vitis*, whereas in our hops, A was reduced by less than 40% after the same decline of g_S.

Without water limitation, the hop cultivar Saaz exhibits relatively high photosynthetic rates that rank to the highest among several hop cultivars (Hejnák et al. [2015]). The maximum photosynthetic rates of the plants in our experiment were equal to those previously observed for this cultivar, despite the approximately 40 ppm lower CO_2 concentration used in our measurements. The effect of globally increasing ambient CO_2 concentrations on photosynthetic rates may not be as strong. However, it is more likely to lead to a greater increase in WUE, as has been found for several other species (Allen et al. [2011]; Liu et al. [2023]).

A well-coordinated adjustment of g_S and A can help to maintain a positive carbon balance under drought. Positive diurnal plant carbon balance and greatly reduced energy requirements for growth may increase the allocation of assimilated carbon to non-structural carbohydrate reserves (Muller et al. [2011]). Increased non-structural carbohydrate reserves may be beneficial for improving plant drought tolerance capabilities (osmotic adjustment, antioxidant activity) as well as during recovery from drought stress [recovery of xylem transport capacity, growth and other physiological functions (McDowell et al. [2008])]. However, the capability of hops to recover from drought stress is, to our knowledge, very poorly understood.

When stomata remained fully closed and both g_S and photosynthetic rate dropped below 10% of maximum, we found a surprisingly high residual TR (about 20% of maximum TR; Fig. [1]), which was higher than in several other angiosperm species (Machado et al. [2021]). Although the value may be slightly overestimated due to less precise gravimetric measurements at the whole plant level, considerably high residual transpiration may represent a serious threat for hop plants under high VPDs when water availability is reduced for a longer period of time. A high risk of extensive water loss was also indicated by our detailed analysis of the daily changes of TR and Ψ_L, which showed a significant decrease of Ψ_L in both treatments towards the afternoon hours. Our data suggest that despite progressive stomatal closure, water loss of WL plants tends to be greater than water supply by xylem transport. The sensitive stomatal response to reduced water availability observed in hop plants cannot efficiently prevent the decrease in tissue water content. The continuous loss of water from the tissues can lead to a rapid decrease in xylem water potential and thus to an increase in embolism formation within a relatively short period of time. The increase in embolism then further complicates shoot water supply.

We found that hop plants operate at water potentials close to Ψ_{12} – where rapid embolization begins (air-entry point). This means that only a small decrease in xylem water potential (e.g. during a decline in water availability) leads to progressive embolism formation. The steep slope of the PLC curve observed in hop plants suggests a very large loss of xylem hydraulic conductivity within a minimal decrease in Ψ_S, which represents a serious risk of critical hydraulic failure once the embolism starts to spread during limited water availability. High xylem vulnerability to embolism spread is typical of many climbing species (Bartlett et al. [2016]), making them particularly sensitive to hydraulic failure among angiosperm species. Xylem in hop stems exhibited moderate to high vulnerability to hydraulic failure ($\Psi_{50} = -1.6$ MPa) compared to other temperate angiosperm broadleaf species, for which Ψ_{50} typically ranges from 0.5 to 8.4 MPa (see Choat et al. [2012] and references therein). There are only few reported examples of more sensitive liana species than the hop plants studied. Young perennial stems of the climber *Hedera helix* showed $\Psi_{50} = -0.75$ MPa (Ganthaler et al. [2019]), while stems of *Vitis vinifera* cv. Thomson Seedless had $\Psi_{50} = -1.29$ MPa (Pouzoulet et al. [2020]). There are, however, several *Vitis* cultivars that show greater resistance of xylem to embolism, such as Chardonnay ($\Psi_{50} = -2.27$ MPa) or Sauvignon Blanc ($\Psi_{50} = -2.63$ MPa) (Alsina et al. [2007]).

Our data indicate that stomatal control in hop is well coordinated with xylem vulnerability. Therefore, the sensitive stomatal closing observed in the studied hop plants appears to be an efficient mechanism to rapidly reduce water loss and prevent the excessive decrease of Ψ_L and Ψ_S under high evaporative conditions. The coordination of stomatal closing and xylem vulnerability is consistent with several previous experiments showing that plants typically close their stomata at stem water potentials roughly corresponding

to Ψ_{12} to avoid extensive embolism formation (Chen et al. 2017; Choat et al. 2018).

Practical implications for field-grown plants

The results of this study suggest that hop plants are sensitive to even small decreases in soil water availability. However, it is important to emphasize that the data presented in this paper were obtained using plants grown in pots. The response of plants in the field may be different in some respects. Namely, the older, well-developed plants can access water in deeper soil layers. Hop plants typically produce large root systems with a rooting volume of 4.1 m^3, accessing a depth of 1.6 m (Graf et al. 2014). This can provide hop plants with a reserve of available water and mitigate the severe continuous decline in shoot water potential even during prolonged dry periods. Therefore, fields with easy access to groundwater, such as river basins, may provide more stability for hop vegetative growth and yield. The water use strategy based on a deep root system has been previously identified in climbing plants, e.g. in some *Vitis* cultivars (Pouzoulet et al. 2020).

However, the permanent availability of water to deeper parts of the root system may not necessarily prevent the decrease of TR during the day under reduced precipitation. The insufficient absorption capacity of the whole root system or the generation of long-distance stress signals in the dry parts of the root system in the upper soil layers may induce sensitive stomatal closing at the early decrease of water availability (Bucci et al. 2009; West et al. 2012). Consistently, additional irrigation was found to significantly promote the yield response of hops (Fandiño et al. 2015; Donner et al. 2020; Potopová et al. 2021), suggesting that young plants in their first season in the field may be particularly sensitive to reduced water availability compared to older plants. Experiments with different irrigation doses in the field also indicated a higher sensitivity of young hop plants to reduced water availability (Nakawuka et al. 2017).

This is the first study specifically focused on the regulatory processes of water transport and use in hop plants. The sensitivity of the stomatal response and the high susceptibility of xylem transport to embolism indicate that hop is unsuitable for water-saving irrigation techniques such as deficit irrigation or partial rootzone drying. Larger daily reductions in irrigation water supply can significantly reduce yields of several recent hop cultivars (Nakawuka et al. 2017). We suggest that for more significant savings of irrigation water with minimal penalty on final yield, the application of optimized targeted irrigation during the most sensitive ontogenetic stages (e.g., stem elongation stage) is a more promising research direction. Future hop breeding strategies should include stomatal responses and xylem resistance to embolism among the traits used to select and breed new hop varieties with improved drought tolerance.

Declarations

Conflict of interests The authors declare no conflict of interest.

References

Acosta-Rangel A, Rechcigl J, Bollin S et al (2021) Hop (*Humulus lupulus* L.) phenology, growth, and yield under subtropical climatic conditions: effects of cultivars and crop management. Aust J Crop Sci 764–772. https://doi.org/10.21475/ajcs.21.15.05.p3192

Ali O, Cheddadi I, Landrein B, Long Y (2023) Revisiting the relationship between turgor pressure and plant cell growth. New Phytol 238:62–69. https://doi.org/10.1111/nph.18683

Allen LH, Kakani VG, Vu JCV, Boote KJ (2011) Elevated CO2 increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum. J Plant Physiol 168:1909–1918. https://doi.org/10.1016/j.jplph.2011.05.005

Alsina MM, Herralde FD, Aranda X et al (2007) Water relations and vulnerability to embolism are not related: experiments with eight grapevine cultivars. Vitis 46:1–6

Baize D (1993) Soil science analyses: a guide to current use. John Wiley, New York

Bartlett MK, Klein T, Jansen S et al (2016) The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. PNAS 113:13098–13103. https://doi.org/10.1073/pnas.1604088113

Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol 132:2166–2173. https://doi.org/10.1104/pp.103.023879

Bucci SJ, Scholz FG, Goldstein G et al (2009) Soil water availability and rooting depth as determinants of hydraulic architecture of Patagonian Woody species. Oecologia 160:631–641. https://doi.org/10.1007/s00442-009-1331-z

Buckley TN (2019) How do stomata respond to water status? New Phytol 224:21–36. https://doi.org/10.1111/nph.15899

Chen Y-J, Schnitzer SA, Zhang Y-J et al (2017) Physiological regulation and efficient xylem water transport regulate diurnal water and carbon balances of tropical lianas. Funct Ecol 31:306–317. https://doi.org/10.1111/1365-2435.12724

Choat B, Jansen S, Brodribb TJ et al (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755. https://doi.org/10.1038/nature11688

Choat B, Brodribb TJ, Brodersen CR et al (2018) Triggers of tree mortality under drought. Nature 558:531–539. https://doi.org/10.1038/s41586-018-0240-x

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees 15:204–214. https://doi.org/10.1007/s004680100095

Domec J-C, Schäfer K, Oren R et al (2010) Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO2 concentration. Tree Physiol 30:1001–1015. https://doi.org/10.1093/treephys/tpq054

Donner P, Pokorný J, Ježek J et al (2020) Influence of weather conditions, irrigation and plant age on yield and alpha-acids content of Czech hop (*Humulus lupulus* L.) cultivars. Plant Soil Environ 66:41–46. https://doi.org/10.17221/627/2019-PSE

Edwards CE, Ewers BE, Williams DG et al (2011) The genetic architecture of ecophysiological and circadian traits in *Brassica rapa*. Genetics 189:375–U1107. https://doi.org/10.1534/genetics.110.125112

Ennajeh M, Tounekti T, Vadel AM et al (2008) Water relations and drought-induced embolism in olive (*Olea europaea*) varieties 'Meski' and 'Chemlali' during severe drought. Tree Physiol 28:971–976. https://doi.org/10.1093/treephys/28.6.971

Fandiño M, Olmedo JL, Martínez EM et al (2015) Assessing and modelling water use and the partition of evapotranspiration of irrigated hop (*Humulus lupulus*), and relations of transpiration with hops yield and alpha-acids. Ind Crops Prod 77:204–217. https://doi.org/10.1016/j.indcrop.2015.08.042

Flexas J, Bota J, Escalona JM et al (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct Plant Biol 29:461. https://doi.org/10.1071/PP01119

Ganthaler A, Marx K, Beikircher B, Mayr S (2019) Are hydraulic patterns of lianas different from trees? New insights from Hedera helix. JExpBot 70:2811–2822. https://doi.org/10.1093/jxb/erz071

Gloser V, Baláž M, Svoboda P (2011) Analysis of anatomical and functional traits of xylem in *Humulus lupulus* L. stems. Plant Soil Environ 57

Gloser V, Balaz M, Jupa R et al (2013) The Response of *Humulus lupulus* to Drought: the Contribution of Structural and Functional Plant Traits. In: Patzak J, Koutoulis A (eds) Acta Horticulturae, Proceedings of III. International Humulus Symposium. Int Soc Horticultural Science, Leuven 1, pp 149–154

Graf T, Beck M, Mauermeier M et al (2014) *Humulus lupulus*-The Hidden Half. BrewingScience 67:161–166

Groenveld T, Obiero C, Yu Y et al (2023) Predawn leaf water potential of grapevines is not necessarily a good proxy for soil moisture. BMC Plant Biol 23:369. https://doi.org/10.1186/s12870-023-04378-6

Hacke U, Sauter JJ (1995) Vulnerability of xylem to embolism in relation to leaf water potential and stomatal conductance in *Fagus sylvatica* f. purpurea and *Populus balsamifera*. J Exp Bot 46:1177–1183. https://doi.org/10.1093/jxb/46.9.1177

Hari V, Rakovec O, Markonis Y et al (2020) Increased future occurrences of the exceptional 2018–2019 central European drought under global warming. Sci Rep 10:12207. https://doi.org/10.1038/s41598-020-68872-9

Hejnák V, Hniličková H, Hnilička F (2015) Physiological response of juvenile hop plants to water deficit. Plant Soil Environ 61:332–338. https://doi.org/10.17221/279/2015-PSE

Hlavinka P, Trnka M, Semeradova D et al (2009) Effect of drought on yield variability of key crops in Czech Republic. Agric for Meteorol 149:431–442. https://doi.org/10.1016/j.agrformet.2008.09.004

Hochberg U, Rockwell FE, Holbrook NM, Cochard H (2018) Iso/Anisohydry: a plant–environment interaction rather than a simple hydraulic trait. Trends Plant Sci 23:112–120. https://doi.org/10.1016/j.tplants.2017.11.002

Hops harvest reports https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/hops/hops-harvest-reports_en. Oct 2023

Jacobsen AL, Pratt RB, Tobin MF et al (2012) A global analysis of xylem vessel length in woody plants. Am J Bot 99:1583–1591. https://doi.org/10.3732/ajb.1200140

Jupa R, Baláž M, Svoboda P, Gloser V (2013) Inherent variability in structural and functional traits of xylem among three hop varieties. Plant Soil Environ 59:273–279

Jupa R, Plavcová L, Flamiková B, Gloser V (2016a) Effects of limited water availability on xylem transport in liana *Humulus lupulus* L. Environ Exp Bot 130:22–32. https://doi.org/10.1016/j.envexpbot.2016.05.008

Jupa R, Plavcová L, Gloser V, Jansen S (2016b) Linking xylem water storage with anatomical parameters in five temperate tree species. Tree Physiol 36. https://doi.org/10.1093/treephys/tpw020

Köcher P, Horna V, Leuschner C (2013) Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and dependence on tree functional traits. Tree Physiol 33:817–832. https://doi.org/10.1093/treephys/tpt055

Koide RT, Robichaux RH, Morse SR, Smith CM (1989) Plant water status, hydraulic resistance and capacitance. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological Ecology: field methods and instrumentation. Springer Netherlands, Dordrecht, pp 161–183

Kolenc Z, Vodnik D, Mandelc S et al (2016) Hop (*Humulus lupulus* L.) response mechanisms in drought stress: proteomic analysis with physiology. Plant Physiol Biochem 105:67–78. https://doi.org/10.1016/j.plaphy.2016.03.026

Korovetska H, Novák O, Jůza O, Gloser V (2014) Signalling mechanisms involved in the response of two varieties of *Humulus lupulus* L. to soil drying: I. changes in xylem sap pH and the concentrations of abscisic acid and anions. Plant Soil 380:375–387. https://doi.org/10.1007/s11104-014-2101-1

Leuzinger S, Hartmann A, Koerner C (2011) Water relations of climbing ivy in a temperate forest. Planta 233:1087–1096. https://doi.org/10.1007/s00425-011-1363-6

Li S, Lens F, Espino S et al (2016) Intervessel pit membrane thickness as a key determinant of embolism resistance in angiosperm xylem. IAWA J 37:152–171. https://doi.org/10.1163/22941932-20160128

Liu W, Liu L, Yan R et al (2023) A comprehensive meta-analysis of the impacts of intensified drought and elevated CO2 on forage

growth. J Environ Manage 327:116885. https://doi.org/10.1016/j.jenvman.2022.116885

Machado R, Loram-Lourenço L, Farnese FS et al (2021) Where do leaf water leaks come from? Trade-offs underlying the variability in minimum conductance across tropical savanna species with contrasting growth strategies. New Phytol 229:1415–1430. https://doi.org/10.1111/nph.16941

Martínez-Vilalta J, Garcia-Forner N (2017) Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ 40:962–976. https://doi.org/10.1111/pce.12846

McDowell N, Pockman WT, Allen CD et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x

Mozny M, Tolasz R, Nekovar J et al (2009) The impact of climate change on the yield and quality of Saaz hops in the Czech Republic. Agric Meteorol 149:913–919. https://doi.org/10.1016/j.agrformet.2009.02.006

Mozny M, Trnka M, Vlach V et al (2023) Climate-induced decline in the quality and quantity of European hops calls for immediate adaptation measures. Nat Commun 14:6028. https://doi.org/10.1038/s41467-023-41474-5

Muller B, Pantin F, Génard M et al (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. J Exp Bot 62:1715–1729. https://doi.org/10.1093/jxb/erq438

Nakawuka P, Peters TR, Kenny S, Walsh D (2017) Effect of deficit irrigation on yield quantity and quality, water productivity and economic returns of four cultivars of hops in the Yakima Valley, Washington State. Ind Crops Prod 98:82–92. https://doi.org/10.1016/j.indcrop.2017.01.037

Nardini A, Salleo S (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? Trees 15:14–24. https://doi.org/10.1007/s004680000071

Nonami H (1998) Plant water relations and control of cell elongation at low water potentials. J Plant Res 111:373–382. https://doi.org/10.1007/BF02507801

Pammenter NW, Vander Willigen C (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. Tree Physiol 18:589–593. https://doi.org/10.1093/treephys/18.8-9.589

Potkay A, Trugman AT, Wang Y et al (2021) Coupled whole-tree optimality and xylem hydraulics explain dynamic biomass partitioning. New Phytol 230:2226–2245. https://doi.org/10.1111/nph.17242

Potopová V, Lhotka O, Možný M, Musiolková M (2021) Vulnerability of hop-yields due to compound drought and heat events over European key-hop regions. Int J Climatol 41:E2136–E2158. https://doi.org/10.1002/joc.6836

Pouzoulet J, Pivovaroff AL, Scudiero E et al (2020) Contrasting adaptation of xylem to dehydration in two *Vitis vinifera* L. sub-species. Vitis 53–61 Pages. https://doi.org/10.5073/VITIS.2020.59.53-61

Qu M, Zheng G, Hamdani S et al (2017) Leaf Photosynthetic parameters related to Biomass Accumulation in a Global Rice Diversity Survey. Plant Physiol 175:248–258. https://doi.org/10.1104/pp.17.00332

Rosell JA (2016) Bark thickness across the angiosperms: more than just fire. New Phytol 211:90–102. https://doi.org/10.1111/nph.13889

Rosell JA, Olson ME, Anfodillo T, Martínez-Méndez N (2017) Exploring the bark thickness–stem diameter relationship: clues from lianas, successive cambia, monocots and gymnosperms. New Phytol 215:569–581. https://doi.org/10.1111/nph.14628

Rossbauer G, Buhr L, Hack H et al (1995) Phänologische Entwicklungsstadien Von Kultur-Hopfen (*Humulus lupulus* L.) Codierung Und Beschreibung Nach Der Erweiterten BBCH-Skala Mit Abbildungen. Nachrichtenbl Deut Pflanzenschutzd 47:249–253

Rybacek V (2012) Hop Production. Elsevier. 229p

Salleo S, Nardini A, Pitt F, Gullo MAL (2000) Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L). Plant Cell Environ 23:71–79. https://doi.org/10.1046/j.1365-3040.2000.00516.x

Scholz FG, Phillips NG, Bucci SJ et al (2011) Hydraulic capacitance: biophysics and Functional Significance of Internal Water Sources in relation to Tree size. In: Meinzer FC, Lachenbruch B, Dawson TE (eds) Size- and Age-related changes in Tree structure and function. Springer Netherlands, Dordrecht, pp 341–361

Sperry J, Donnelly J, Tyree M (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11:35–40. https://doi.org/10.1111/j.1365-3040.1988.tb01774.x

Sperry JS, Meinzer FC, McCulloh KA (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant Cell Environ 31:632–645. https://doi.org/10.1111/j.1365-3040.2007.01765.x

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of Sap. Springer, Berlin, Heidelberg

West AG, Dawson TE, February EC et al (2012) Diverse functional responses to drought in a Mediterranean-type shrubland in South Africa. New Phytol 195:396–407. https://doi.org/10.1111/j.1469-8137.2012.04170.x

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in Woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. Plant Cell Environ 28:800–812. https://doi.org/10.1111/j.1365-3040.2005.01330.x

Yang Y-J, Bi M-H, Nie Z-F et al (2021) Evolution of stomatal closure to optimize water-use efficiency in response to dehydration in ferns and seed plants. New Phytol 230:2001–2010. https://doi.org/10.1111/nph.17278

Zweifel R, Sterck F, Braun S et al (2021) Why trees grow at night. New Phytol 231:2174–2185. https://doi.org/10.1111/nph.17552