RESEARCH ARTICLE

Soil water availability modulates the response of grapevine leaf gas exchange and PSII traits to a simulated heat wave

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Abstract

Background and aims A better understanding of plant carbon assimilation, water status and photosystem performance responses to combined heat and drought stress would help to optimize grapevine management under such limiting conditions.

Methods Gas exchange and chlorophyll fluorescence parameters were measured in potted grapevines, cv Sauvignon Blanc, before, during and after simulated six-day heat ($T_{max} = 40$ °C) wave using heated well-watered (HW), heated drought-stressed (HD), non-heated well-watered (CW) and non-heated dry (CD) vines.

Results Photosynthesis and stomatal conductance in HW vines increased during the morning and dropped in the afternoon with respect to CW vines. Daily plant transpiration in HW almost doubled that of CW vines. When grapevines were already exposed to drought, the effects of the heat wave were negligible, with HD plants showing similar leaf photosynthesis and transpiration to their CD counterparts. Heat, but

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not drought stress, decreased the maximum (Fv/Fm) and effective photochemical quantum yield of PSII (φPSII), and also affected the use of absorbed energy. HW plants dissipated more radiative energy as heat, a protective mechanism of the photosystem, while HD vines increased the energy dissipated by non-regulated non-photochemical pathways, which might lead to photoinhibition damages. The different behavior could be due to the enhanced transpiration rate and consequent decrease in leaf temperature in HW as compared to HD vines. After the heat wave, only HW vines recovered the afternoon values of photosynthesis, stomatal conductance and φPSII to similar levels as those in CW vines.

Conclusion Drought had a more significant effect than heat stress on photosynthesis, stomatal conductance and transpiration. The combined heat and drought stress, however, increased the proportion of energy lost by the leaves through harmful non-regulated dissipative pathways. With adequate soil water availability, grapevines withstood the heat wave period through an increase in leaf transpiration, which decreased leaf temperature and protected the PSII from heat damage.

Highlights Drought had a stronger impact on gas exchange parameters than elevated temperature during a simulated heatwave, while heat stress was the main driver of PSII functionality and absorbed energy partitioning. Well-watered grapevines were able to recover their physiological function after a six-day heatwave (T_{max} 40 °C), while plants under heat and drought stress were unable to resume PSII performance after one day of recovery.

Keywords Chlorophyll fluorescence · Energy partitioning · Drought stress · Gas exchange · Grapevines · Heat stress · Heat waves · Multiple stressors · Plant transpiration

Abbreviations

C	Control temperature treatment
CD	Plants under control temperature and water-stress treatment
CW	Plants under control temperature and well-watered treatment
D	Water-stress treatment
Fv/Fm	Maximum photochemical quantum yield of photosystem II
g_s	Stomatal conductance to water vapor
H	Heat treatment
HD	Plants under heat and dry treatment
HW	Plants under heat and well-watered treatment
P_n	Net assimilation rate
PSII	Photosystem II
Ψ_{stem}	Stem water potential
Ψ_{soil}	Soil water potential
SWC	Soil water content
E_l	Leaf transpiration rate
E_p	Whole-plant transpiration rate
T_{max}	Maximum daily air temperature
T_{min}	Minimum daily air temperature
VPD	Vapor pressure deficit
W	Well-watered treatment
φPSII	Effective photochemical quantum yield of photosystem II
Y(NO)	Quantum yield of light-independent non-photochemical fluorescence quenching
Y(NPQ)	Quantum yield of light-dependent non-photochemical fluorescence quenching

Introduction

Grapevines are traditionally cultivated in areas with long growing seasons, often characterized by more than 182 days with mean temperatures above 10 °C (Jackson 2001), which roughly corresponds to the belt limited by the 10 °C to 20 °C annual mean isotherms (Spellman 1999). According to the Intergovernmental Panel on Climate Change 2021 report, heat waves and drought events have been increasing in frequency since 1950, with higher intensity and duration due to climate change (Arias et al. 2021). Recently, NASA reported record-breaking temperatures in the summer of 2022 due to heat waves across Europe, North Africa, the Middle East and Asia, with temperatures exceeding 40 °C for extended periods of time in certain areas (NASA 2022).

Summer heat waves are frequently accompanied by drought (Mukherjee and Mishra 2021). Under such conditions, grapevines will likely be exposed to multiple stressors (i.e. high temperature and low water availability) that may negatively impact plant growth, yield and berry quality (Keller 2020).

Separating the effects of heat from drought stress on plant physiology is hard to accomplish when the two stressors occur contemporaneously, so factorial experiments under controlled conditions are key for unravelling plant responses to combined stresses. Carvalho and Amâncio (2019) reviewed the physiological (gas exchange, chlorophyll fluorescence and stem water potential) and metabolic (electrolyte leakage, C/N ratio, primary and secondary metabolism) responses of grapevines to abiotic stresses and identified common responses to single and combined heat and drought stress, such as the decrease in photosynthesis, the accumulation of antioxidants and the shift from primary to secondary metabolites. Plant physiological responses to combined stresses, however, can be non-additive due to the induction of synergistic or antagonistic mechanisms (Rizhsky et al. 2002; Carvalho and Amâncio 2019).

It is still unclear whether the combination of heat and drought stress has higher negative effects on plant growth and productivity than the sum of the effects of the single stress factors. Similarly, only a few attempts have been made so far to investigate which of the stresses has a stronger impact in determining plant physiological responses (Edwards et al. 2011; Galat-Giorgi et al. 2019; Tan et al. 2023). This is likely because of the multiple and interconnected endogenous and environmental variables that drive plant physiology. When limited soil water availability causes leaf water potentials to become too negative, plants typically close their stomata in order to avoid losing further water, which reduces transpiration, photosynthesis and plant growth (Redondo-Gómez 2013; Dewar et al. 2018; Galat-Giorgi et al. 2019). The reduction in transpiration, in turn, limits the plant's ability to control leaf temperatures via evaporative cooling. Maintaining leaf temperatures within the optimal photosynthetic range (Venios et al. 2020) is critical, because photosystem II (PSII) is highly sensitive to heat stress and can be partially or completely inhibited before visual stress symptoms appear on leaves (Liu et al. 2012; Kalaji et al. 2017). Zha et al. (2019) observed heat injury symptoms in table grapes after a few hours of exposure to 45 °C, with damages to the photosystem II indicated by a significant decrease in minimal fluorescence F_0 compared to non-heated plants. Kadir et al. (2007) also found that a gradual exposure to heat stress causes less damage to the photosystem of grapevines than a sudden exposure to high temperature. Thus, when water deficit and high temperatures are combined, the outcome will depend on the severity, timing and duration of the stress.

The modulation of stomatal conductance represents a first important physiological response of grapevines when exposed to single and combined stressors. The intensity and velocity of stomatal closure partially depend on grapevine genotypes (cultivars), reflecting differences in plant water-use strategies (Gambetta et al. 2020). Some grapevine cultivars are more conservative; they close their stomata soon after the leaf water potential starts to decrease. In contrast, other cultivars keep their stomata open despite relatively low leaf water potential, allowing for more effective heat dissipation through transpiration (Villalobos and Fereres 2016; Gutiérrez-Gamboa et al. 2019; Venios et al. 2020). Against the combined effect of drought and heat, maintaining the stomata open might help control leaf temperature through evaporative cooling, thus maintaining relatively high photosynthetic rates, as demonstrated in the stress-tolerant Malbec grapevine (Galat-Giorgi et al. 2019).

We hypothesize that when subjected to drought and heat, grapevine leaves are mainly affected by the drought component because of their tight stomal regulation. Therefore, we would not expect differences in g_s and Ψ_{stem} between combined heat and drought (HD) and single drought treatments (CD). We further hypothesize that when subjected to combined heat and drought stressors (HD), the grapevine leaves will experience an additional negative effect, decreasing photosynthesis and chlorophyll fluorescence traits further when compared to the single heat (HW) and drought (CD) stress treatments. The relatively few studies exploring the stress effects of combined heat and drought on grapevines support these hypotheses (Edwards et al. 2011; Lehr 2021; Tan et al. 2023), although the link between these observations and the photosystem performance is still not well understood. In addition, some of these experiments were performed with small potted plants (5 L pots) and the heat stress was applied over relatively short periods (48 h, Edwards et al. 2011; Tan et al. 2023), so it remains unclear if the same responses would be observed under more realistic conditions. In order to narrow this knowledge gap and to improve our understanding of the processes behind plant physiological responses (leaf gas exchange, chlorophyll

fluorescence, whole plant transpiration and stem water potential) to combined heat and drought stress, we conducted a factorial experiment simulating a heat wave under controlled conditions and measured gas exchange and chlorophyll fluorescence parameters on Sauvignon Blanc, a grapevine variety with tight stomatal control (Gutiérrez-Gamboa et al. 2019; Gambetta et al. 2020). The objectives were (i) to disentangle the effects of high temperatures from drought on grapevine physiology during a heat wave event and ii) to investigate the effects of soil water availability on the recovery of the vines after a heat wave.

Material and Methods

Plant material and experimental design

Thirty single-node *Vitis vinifera L.* cv. Sauvignon Blanc plants grafted on SO4 rootstock cuttings were planted in 30 L pots filled with a sandy silt soil at the Laimburg Research Centre in the winter of 2019–20. Soil characteristics are listed in Supplementary Table S1. The plants were grown in a greenhouse during the 2020 growing season until March 2021. Vines were then transplanted into 50 L pots and stored in a refrigeration cell at 6 °C. The heat wave simulation experiment was performed in two trials, separated by two weeks (see below). In order to avoid differences in the phenological stage of the two groups of plants when entering the climatic chambers, we delayed the physiological development of the second batch of plants (second trial) by keeping them for a longer period in the refrigeration cell at 6 °C. Thus, the first group of plants was transferred from the cold cell to the greenhouse on 31 March, while the second group entered the greenhouse three weeks later, on 21 April. Bud burst started on approximately the ninth day after moving the plants from the refrigeration cell to the greenhouse for both plant groups. Shoot thinning was performed to allow the presence of four shoots per plant, and inflorescences were removed to enhance homogeneity among plants, as well as among plant shoots on each individual plant.

The factorial experiment had a split-plot design with the first treatment represented by temperature (main plot) with two levels (heat and control: H, C) and a second represented by irrigation (subplot) with two levels (well-watered and drought-stressed:

W, D). We used six fully controlled environmental chambers (https://terraxcube.eurac.edu/structure/, 2.8 m × 3 m × 2.8 m) with each chamber randomly assigned to one temperature level, and with four plants placed in each chamber, randomly assigned to the two irrigation levels (2 W and 2 D). The study was performed in two trials: in the first trial (12 to 24 July) eight plants from the first group were used. Four were placed in a heat chamber and four in a control chamber. In the second trial (26 July to 7 August) 16 plants (second greenhouse group) were similarly divided and placed in two heat and two control chambers. After placing the vines inside the growth chambers, the primary shoots were pruned to a height of around 150 cm.

Heat wave simulation

In each chamber, we ran 13-day experiments where we either simulated a heat wave or mimicked the 10-year average climatic conditions during the month of July, measured at the weather station located at Laimburg Research Centre (46°22′49″ N, 11°17′10″ E, 224 m asl). The duration of the simulated heat wave was based on the analysis of heat wave characteristics in the area (Zanotelli et al. 2022). Day and night lengths were 16 and 8 h, respectively. Relative humidity min/max were 30/60% for day and night, respectively, while ambient CO_2 concentration was 420 ppm. Radiation intensity and air temperature peaked from 12:00 to 14:00, and from 15:00 to 17:00, respectively (Supplementary Fig. S1). The artificial light mimicked the solar spectrum from 280 to 900 nm, and the maximum photosynthetically active radiation reaching the plants at 2 m height was 1500 µmol m^{-2} s^{-1}. Air temperature and humidity were recorded once every minute; the maximum rate of change for temperature was ± 0.5 °C min^{-1} and 0.4/0.8% min^{-1} (cooling/ heating) for humidity. In the control chambers, the minimum and maximum daily temperatures (T_{min} and T_{max}) were 17 °C and 30 °C for the entire period (Supplementary Fig. S1). In the heat chambers, the temperature started to increase gradually on day 4 until day 6, when T_{max} reached 40 °C and T_{min} 24 °C. This temperature was maintained for 6 days (from day 6 to 11, hereon after referred to as "peak of the heat wave"). On day 12 (referred as to "recovery") the temperature in the heat chambers decreased and the temperature regime

returned to the same as the control chamber (Supplementary Fig. S1).

Irrigation treatment

Half of the plants were well-watered (W), while the other half were subjected to drought stress (D). Every day, from 16:30 to 17:30 and always after the physiological measurements, W plants received the same amount of water they had lost during the previous 24 h (see *Plant transpiration* section below). Drought stress treatment plants received the same amount of irrigation water as the well-watered plants on day 1; then they were irrigated on day 3 with 50% of the water they transpired from day 1. On days 6 and 8 they also received a cumulative amount of irrigation water equal to 50% of the water losses since the previous irrigation on day 3 to prevent an excessive decrease in soil water availability (Supplementary Table S2).

Soil water potential and soil temperature

Soil water potential (Ψ_{soil}) and soil temperature (ST) were measured every 15 min in each pot using Teros 21 and ECH20 10HS sensors (Meter, Munich, Germany) and SMT-100 sensors (UGT, Müncheberg, Germany), respectively. All probes were inserted horizontally inside the pots at 25 cm depth at the beginning of the experiment.

Plant transpiration

Plant transpiration (E_p) was measured gravimetrically using weighing lysimeters (UGT, Müncheberg, Germany) that recorded the pot weight continuously (one-minute intervals). Daily whole plant transpiration was calculated as the difference between the pot weight measured in 24-h intervals, normalized by total leaf area measured at the end of the trial and expressed as L m^{-2} day^{-1}. The daily pattern of whole plant transpiration was calculated by considering the weight differences every hour. To prevent soil evaporation, the soil surface of each pot was covered with a plastic cloth and aluminum foil, which were temporarily removed when irrigation water was applied.

Leaf gas exchange, chlorophyll fluorescence and stem water potential

Measurements of leaf gas exchange were performed on healthy fully expanded leaves (one leaf per plant) inserted between the 6th and 8th node from the base on primary shoots, on days 3, 4, 6, 8, 11 and 12, twice per day: late in the morning (10:00 to 12:00) and in the afternoon (15:00 to 17:00). Leaf net photosynthesis (P_n), stomatal conductance (g_s) and transpiration (E_l) were measured using a portable infrared gas analyzer (GFS-3000, Heinz Walz GmbH, Munich, Germany) connected to a standard measuring head (3010S, maximum enclosed leaf area 8 cm^2). Conditions in the leaf cuvette were set to a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}, a CO_2 mole fraction of 450 μmol mol^{-1} and an airflow rate of 750 μmol s^{-1}. Cuvette temperature was set to follow ambient values.

Dark and light chlorophyll fluorescence measurements were performed together with gas exchange measurements using a portable fluorometer (MINI-PAM *M-Series,* Heinz Walz GmbH, Munich, Germany) on adjacent leaves on days 6, 11 and 12, in the afternoon (15:00 to 17:00). The maximum photochemical quantum yield of photosystem II (Fv/Fm), which represents the maximum efficiency of the photosystem, was measured on leaves that were dark-adapted for 30 min., then the effective photochemical quantum yield of photosystem φPSII was measured after fluorescence reached its steady-state under actinic light set at 450 μmol m^{-2} s^{-1}. Saturating pulse intensity was set to level 9 out of 10 for dark and light fluorescence measurements. The quantum yield of light-dependent and light-independent non-photochemical fluorescence quenching (Y(NPQ) and Y(NO); respectively) were calculated according to Kramer et al. (2004). These parameters represent the energy dissipated as heat through regulated (Y(NPQ)) and non-regulated (Y(NO)) energy dissipation pathways.

Stem water potential (Ψ_{stem}) was measured between 15:00 and 17:00 on two fully expanded, healthy primary shoot leaves per plant on days 3, 5, 8, 10 and 12 in each experimental cycle. Before the measurement, the leaves were enclosed in a plastic bag and covered with aluminum foil for one hour in order to prevent leaf transpiration and allow the equilibration of water potential with the stems. All

measurements were done with a Scholander pressure chamber (Model 3115, Soil Moisture Equipment Corp, Santa Barbara, CA, Unites States).

Biometric measurements and leaf chlorophyll concentration

At the end of the experiment, total leaf area and the length of lateral (secondary) shoots were measured in all plants. Leaf area was measured using a leaf area meter coupled with a transparent belt conveyer (LI-3000C+LI-3050C, LI-COR Biosciences, Lincoln, NE, Unites States).

Leaf samples were collected on day 13 of the experiment to determine total chlorophyll concentration using colorimetric methods as described by Wellburn (1994), with some modifications. Two leaf discs (1 cm^2 / disc) were sampled, covered with aluminum foil and stored at -80 °C until analyses. Discs with 10 ml of dimethyl sulfoxide (VWR International Srl, Milano, IT) were incubated in the dark at 70 °C for 45 min. The absorbance of the extracts was measured at 665, 649 and 480 nm against a blank (dimethyl sulfoxide) using an Agilent Cary 100 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Pigment concentrations were calculated using the equations described in Wellburn (1994) and expressed in µg of pigment mg^{-1} leaf dry weight.

Statistical analyses

All statistical analyses were performed using R software version 4.1.2 (2021–11-01) (R Core Team 2021). Data collected on each day of measurement (leaf gas exchange, chlorophyll fluorescence, whole plant transpiration, leaf and soil water potential) were subjected to analysis of variance carried out by linear mixed-effects models adapted for split-plot designs with "trial" and "chamber" as random factors, using the "lmer" function of the *lme4* R package v. 1.1–27.1 (Bates et al. 2015). Only data from the second trial was used in fluorescence analyses. Morning and afternoon data were analyzed separately in all variables. Non-normally distributed variables (Shapiro–Wilk normality test, $P < 0.05$) were normalized using logarithmic (Pn, gs, E$_l$ and Ψ$_{stem}$) or box-cox transformations (Fv/Fm, φPSII, Y(NPQ), Y(NO)). When the interaction between treatments was significant, the analyses were run using only one fixed factor at a time

(temperature or irrigation) to test for the effect of the different levels of the other treatment. Comparisons within a factor were analyzed using Tukey post-hoc tests, using the *emmeans* R package v. 1.7.1–1 (Lenth 2018). The subset of data collected during the peak of the heat wave (days 6–11) was tested for the cumulative effect of the heat wave (T$_{max}$ 40 °C) on leaf gas exchange both in HW and HD plants, using mixed linear models for repeated measures with irrigation and day as fixed factors, and chamber and plant ID as random factors. A mixed linear model including the last day of the heat wave (day 11) and the day after the heat wave (day 12) was performed to check the effect of the sudden decrease in ambient temperature (T$_{max}$ from 40 °C to 30 °C) on leaf gas exchange and chlorophyll fluorescence variables. Photosynthesis, stomatal conductance and transpiration data were plotted against leaf-to-air VPD (VPD$_{la}$) and the trend lines were smoothed using the 'geom_smooth' function of the *ggplot2* package (method = 'loess') in R. For all data, significance was assumed whenever $P < 0.05$.

Results

Climatic data and soil water availability

During the peak of the heat wave (days 6 to 11, Supplementary Fig. S1), the maximum air temperature reached 40 °C. During this period, vapor pressure deficit (VPD) reached values higher than 5 kPa. Maximum daily air temperature in the control chambers was constant at 30 °C throughout experiment, with maximum VPD values of 3 kPa (Supplementary Fig. S1). Maximum soil temperature in the heat chambers was also 10 °C higher than in control chambers, with T$_{max}$ around 38 °C during the heat wave (Supplementary Fig. S2).

The heat treatment did not affect Ψ$_{soil}$ (Fig. 1), while the drought treatment significantly decreased Ψ$_{soil}$ from day 4 until the end of the experiment (Fig. 1). Ψ$_{soil}$ in well-watered plants ranged from 0 to -400 kPa throughout the entire experiment, corresponding to a volumetric soil water content of between 25 and 30% (Supplementary Fig. S3). Ψ$_{soil}$ in dry plants progressively decreased until -1000 kPa at the end of the experiment (corresponding to 15% volumetric soil water content, Supplementary Fig. S3).
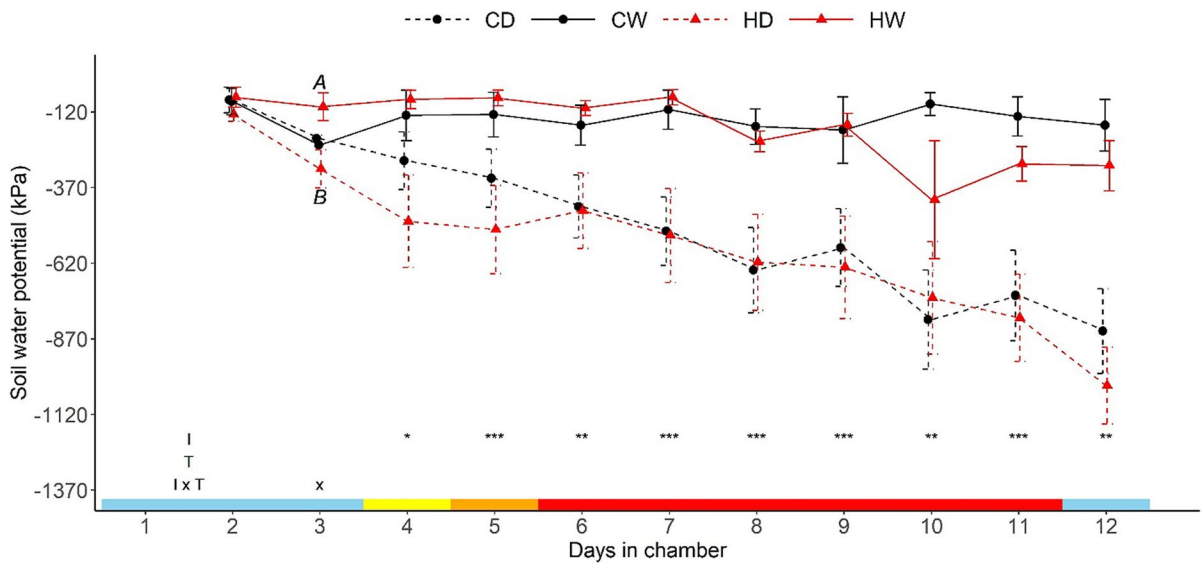
Fig. 1 Soil water potential in control/heat treatments (black/red) and well-watered/drought treatments (solid/dashed) during the experiment. Values are means ± SE. Significant effects of irrigation (I), temperature (T) and their interaction (I x T) within each day are indicated with the symbols *, +, x, respectively. The number of times symbols are reported represents a significance level with $P < 0.05$ (once), < 0.01 (twice), or < 0.001 (three times). Different letters with the same font type indicate significant differences between treatments within the same day: capital letters in italic indicate significant effect of irrigation on plants in heat treatment. The color scale on the bottom indicates the evolution of T_{max} during the heat wave simulation: blue, $T_{max} = 30$ °C; yellow, $T_{max} = 33$ °C; orange, $T_{max} = 37$ °C and red, $T_{max} = 40$ °C

Stem water potential

The dry plants had significantly lower stem water potential (Ψ_{stem}) than well-watered plants from day 3 until the end of the experiment (Fig. 2). The effect of the heat treatment on Ψ_{stem} varied throughout the experiment and had opposite effects on well-watered and dry plants: on days 8 and 10 (peak of the heat wave) the heat treatment significantly increased Ψ_{stem} in HD plants compared their CD counterparts, while it decreased in HW plants compared to their CW counterparts (interaction irrigation x temperature $P < 0.001$, Fig. 2). Ψ_{stem} in CD plants was 68% lower than in CW plants from day 3 to day 12 (-0.96 ± 0.02 and -0.57 ± 0.02 MPa respectively, average period days 3 to 12). Ψ_{stem} in HD and HW plants was not statistically different from day 5 to day 10 and became more negative with the time of heat exposure (42% decrease from -0.59 ± 0.06 MPa on day 5 to -0.84 ± 0.012 MPa on day 10, values are averages of HD and HW plants on day 5 and 10 respectively, Fig. 2). On day 12 (recovery to control temperature), Ψ_{stem} increased in HW plants to reach values similar

to those of CW plants (-0.61 ± 0.02 MPa), while in HD plants, the values dropped to -1.25 ± 0.04 MPa, the lowest values recorded during the experiment.

Whole plant transpiration

Drought significantly reduced whole-plant transpiration regardless of the temperature treatment (55% and 74% reduction in control and heat treatments respectively, on average from days 6 to 11, Fig. 3). E_p in CW plants varied little throughout the experiment (0.89 ± 0.05 L m^{-2} day^{-1} average for the whole experiment). There was a two-fold increase in the transpiration of HW plants during the peak of the heat wave period as compared to CW plants (from 0.95 to 1.82 L m^{-2} day^{-1}, on average from day 6 to 11, Fig. 3), followed by a statistically significant decrease (30%) in response to the decrease in temperature from days 11 to 12 (return to control temperature). The effect of heat on transpiration of HD plants was only marginal. There was a slight increase in E_p in D plants on day 9 due to the small water supply the previous day (Supplementary Table S2). The daily pattern of E_p during
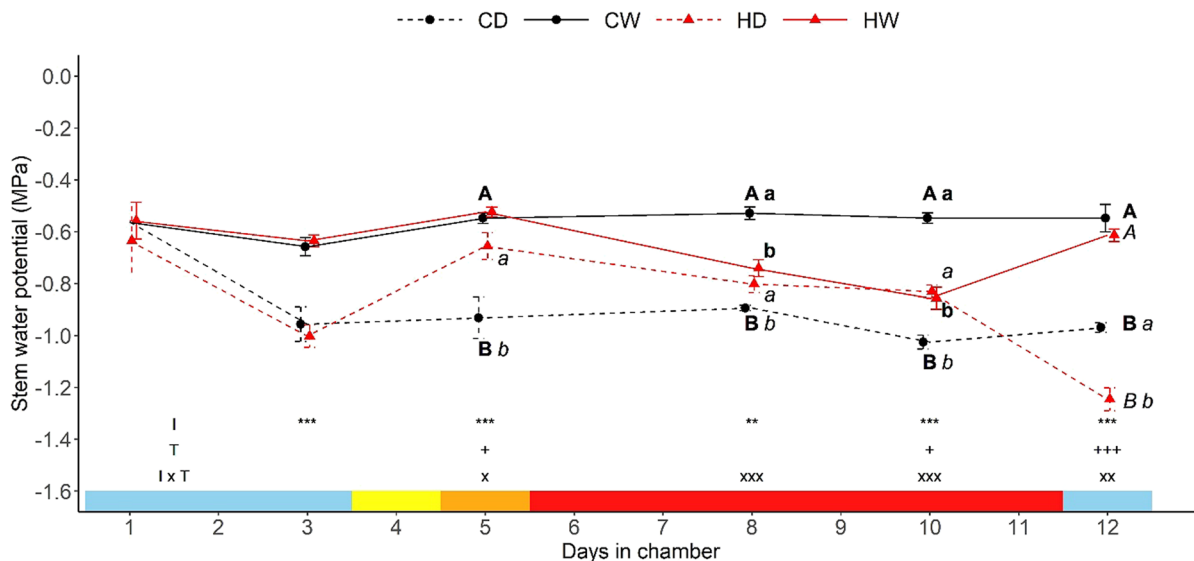
Fig. 2 Stem water potential in control/heat treatments (black/red) and well-watered/drought treatments (solid/dashed). Values are means ± SE. Significant effects of irrigation (I), temperature (T) and their interaction (I x T) within each day are indicated with the symbols *, +, x, respectively. The number of times symbols are reported represents a significance level with $P < 0.05$ (once), < 0.01 (twice), or < 0.001 (three times). Differ-ent letters with the same font type indicate significant differences between treatments within the same day: capital letters in bold and in italic indicate significant effect of irrigation on plants in control and heat treatments, respectively. Small letters in bold and italic indicate significant effect of temperature on water and dry plants, respectively
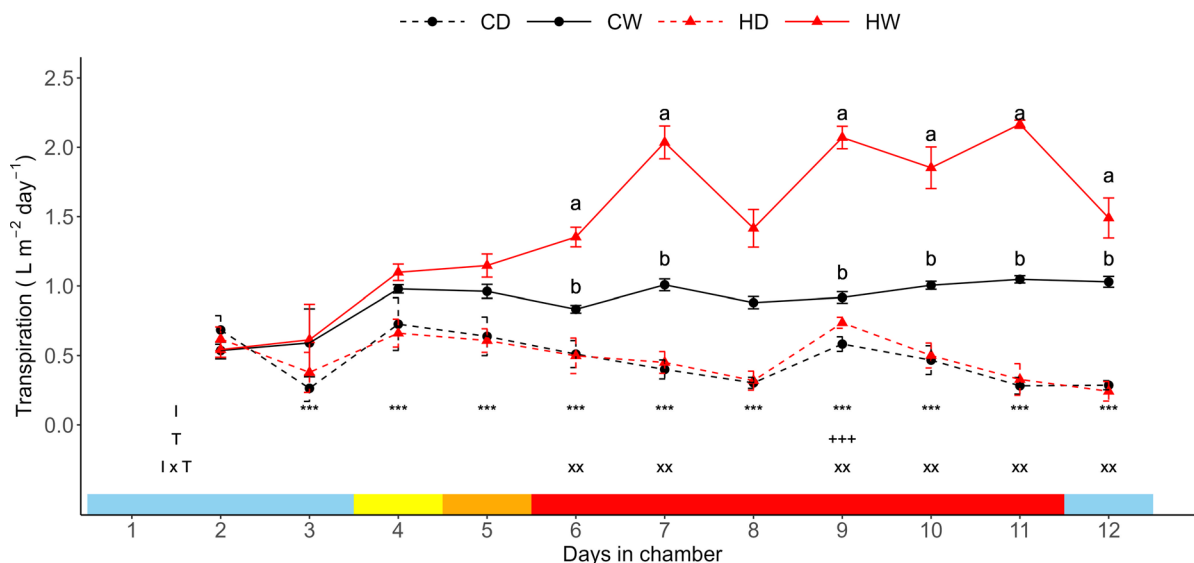

Fig. 3 Whole plant daily transpiration (T_p) in control/heat treatments (black/red) and well-watered/drought treatments (solid/dashed). Values are means ± SE. Significant effects of irrigation (I), temperature (T) and their interaction (I x T) within each day are indicated with the symbols *, +, x, respectively. The number of times symbols are reported represents a significance level with $P < 0.05$ (once), < 0.01 (twice), or < 0.001 (three times). Different letters indicate significant differences between treatments within the same day

the first and last days of the heat wave (days 6 and 11, Fig. 4) showed that morning transpiration accounted for the increased E_p rate of HW plants. There was a recovery in E_p rate from 16:00 to 18:00 in all treatments, just after irrigation (Fig. 4). As a result of the differential transpiration between HW and HD plants during the peak of heat wave, leaf temperature was on average 3.5 ± 0.4 °C (mean $\pm$ standard error) lower in well-watered plants compared to their dry counterparts (Supplementary Fig. S4).

Leaf photosynthesis, transpiration and stomatal conductance

The drought treatment significantly decreased P_n by 78% on average during the whole experiment (average well-watered plants versus dry plants, Fig. 5A). The heat treatment decreased P_n on days 5, 6, and 8 only in the afternoon, in both D and W plants (57% decrease in the heat treatment, averaging dry and well-watered plants, days 5, 6 and 8). The only significant interaction between heat and irrigation treatments was recorded in the morning of day 11, with higher P_n in HWplants than CW plants, but no temperature effect on D plants. There was a decline in P_n in all treatments both in the morning and afternoon

from days 3 to 5, possibly indicating an acclimation period. During the peak of the heat wave (days 6 to 11) the heat treatment did not significantly affect P_n in the morning, despite P_n in HW plants tending to be higher than CW plants, and to increase progressively with the time exposed to heat (from 7.97 ± 0.72 to 11.46 ± 1.05 μmol m^{-2} s^{-1} days 6 to 11, Supplementary Fig. S5A). In this period, the decline of P_n from morning to afternoon in HW plants was larger than in CW plants (73% vs. 28% decline, day period x temperature $P < 0.0001$, Fig. 5A). When temperature returned to control conditions ($T_{max} = 30$ °C, day 12), P_n recovered in the afternoon, although the magnitude of the recovery was higher in HW than in HD plants (Supplementary Fig. S6A).

The drought treatment decreased E_l by 74% on average during whole the experiment (average of well-watered plants versus dry plants, Fig. 5B). The heat treatment increased E_l in HW but not in HD plants, especially in the morning and during the peak of the heat wave. During this period, E_l measured in the morning in HW plants increased with time of exposure to heat (from 2.30 ± 0.19 to 3.11 ± 0.30 mmol m^{-2} s^{-1} days 6 to 11, Supplementary Fig. S5B) and decreased significantly (by 64%) when the temperature returned to control (day 12,
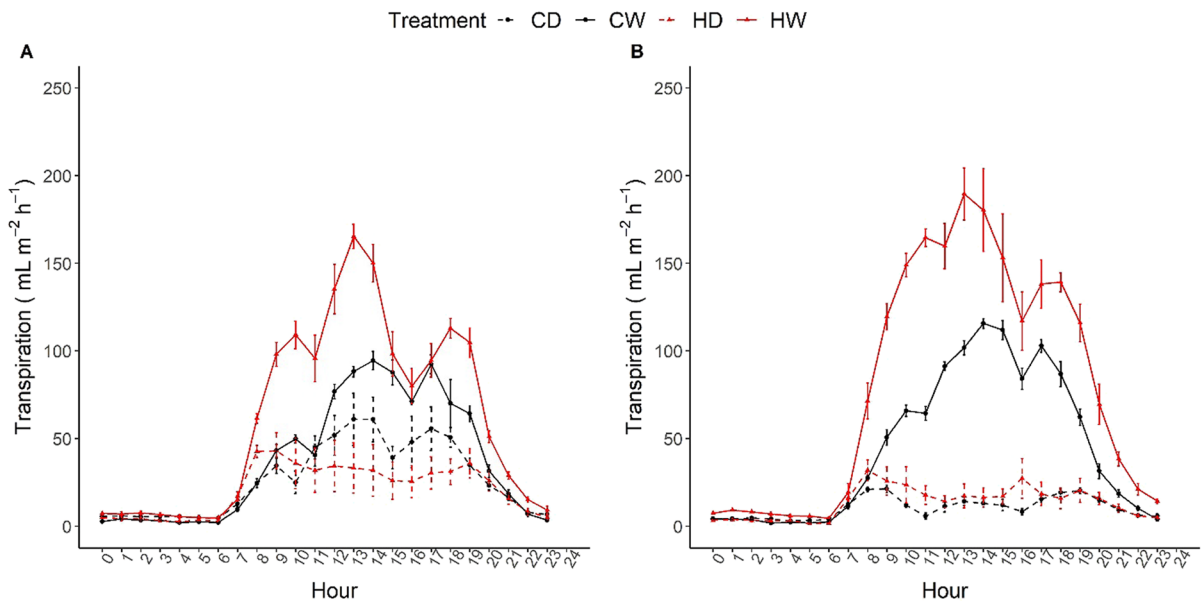

Fig. 4 Daily pattern in hourly whole plant transpiration rate at **(A)** the beginning and **(B)** end of the heat wave (day 6 and 11 respectively) in control/heat treatments (black/red) and well-watered/drought treatments (solid/dashed). Values are means $\pm$ SE ($n = 4$–6)

Fig. 5 (A) Net assimilation, **(B)** leaf transpiration and **(C)** stomatal conductance in control/heat treatments (black/red) and well-watered/drought treatments (solid/dashed) measured in the morning (10 am – 12 pm) and afternoon (3 pm – 5 pm). Values are means ± SE. Significant effects of irrigation (I), temperature (T) and their interaction (I x T) on gas exchange variables within each day are indicated with the symbols *, +, x, respectively. The number of times symbols are reported represents a significance level with $P < 0.05$ (once), < 0.01 (twice), or < 0.001 (three times). Different letters with the same font type indicate significant differences between treatments within the same day: capital letters in bold and in italic indicate significant effect of irrigation in control and heat treatments, respectively. Small letters in bold and italic indicate significant effect of temperature on well-watered and drought treatments, respectively
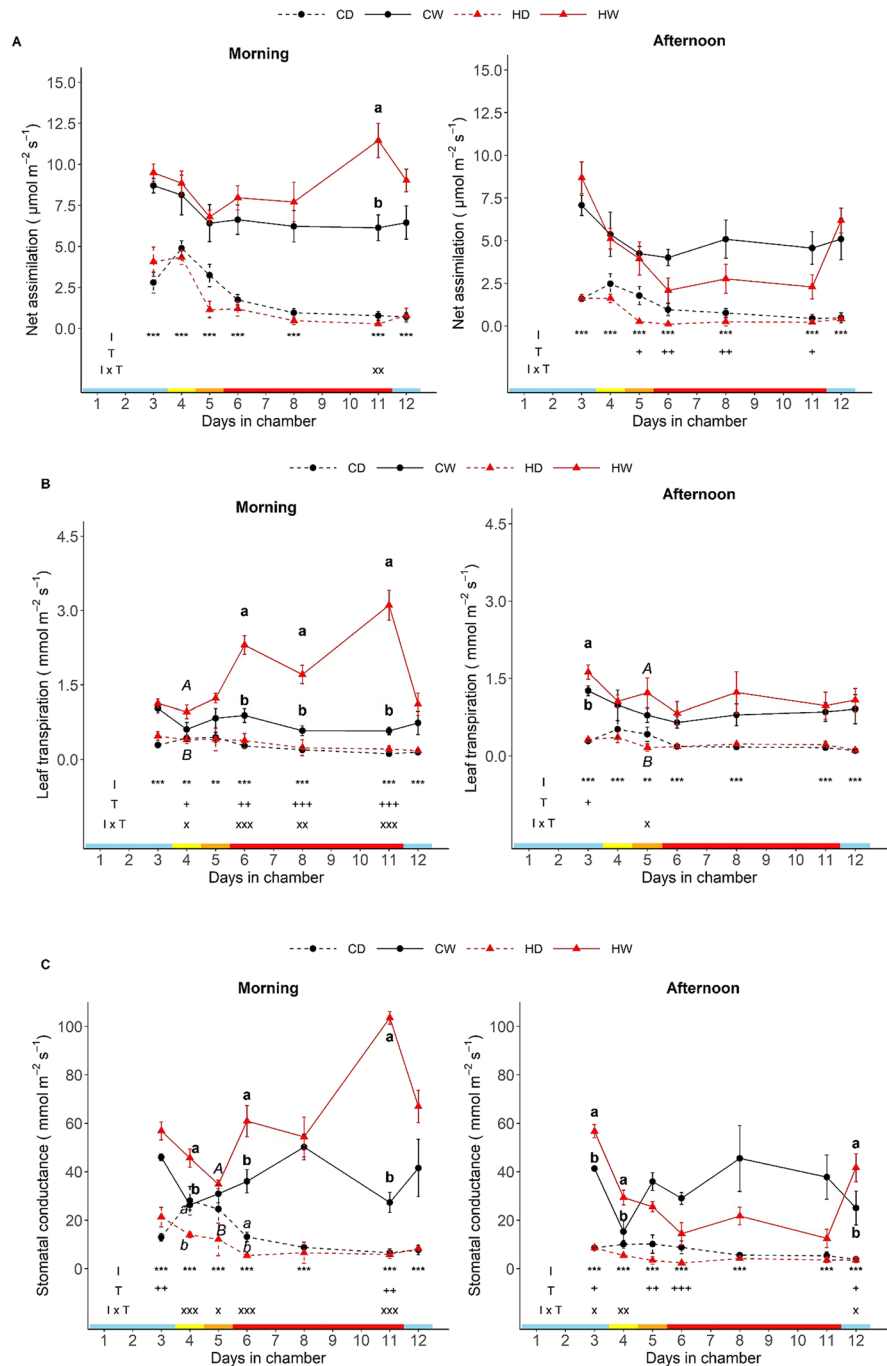
Supplementary Fig. S6B). Conversely, E_l in HD plants remained constant and close to zero. Leaf transpiration during the maximum temperature period of the heat wave decreased significantly from morning to afternoon in HW plants, but not in HD plants.

Stomatal conductance was always significantly lower in dry than in well-watered plants (76% decrease on average, Fig. 5C). During the rise of the heat wave (days 4 to 6) g_s was lower in HD plants than in CD plants, both in the morning and afternoon (57% difference on average). This trend disappeared

Fig. 6 Correlation between leaf photosynthesis (P_n), transpiration (E_l) and stomatal conductance (g_s) and leaf-to-air vapor pressure deficit (VPD_{la}) in dry (a,c,e) and well-watered (b, d, f) plants. Values are means $\pm$ SE for each treatment (control dry, control heat, heat dry, heat water) and period of the day (morning/afternoon, blue and yellow respectively). The size of the dots indicates the corresponding average value of soil water potential. The black line indicates the predicted values obtained by the smoothing method "loess". The grey shaded area indicates the 95% confidence interval around the smooth
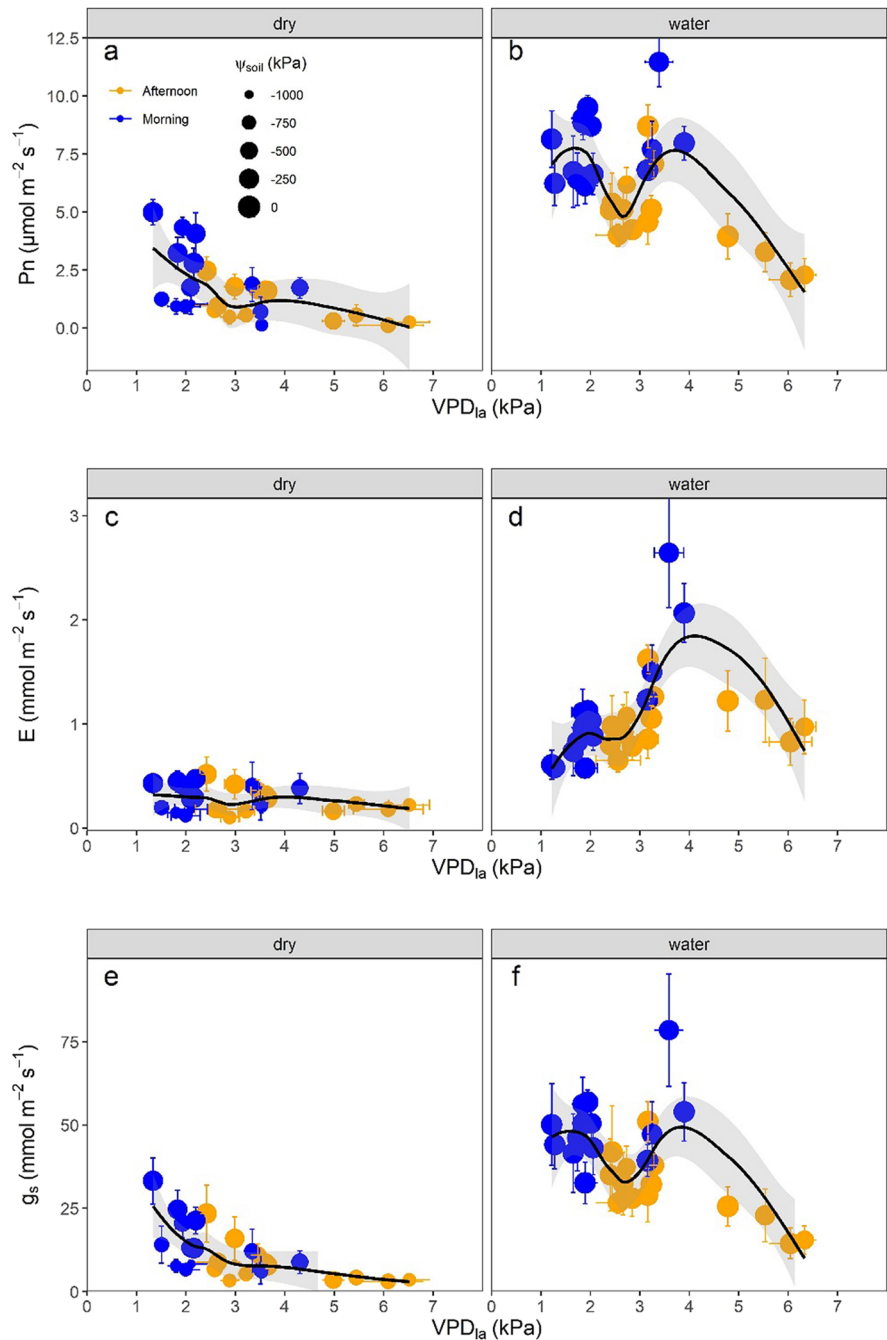
after day 6, when g_s in CD and HD plants was similar and very low, both in the morning and afternoon (range from 3.5 to 8.7 mmol m^{-2} s^{-1} from day 7 to 12 in CD and HD plants, Fig. 5C). On the other hand, g_s in HW plants was higher in the morning than in CW plants, with values that increased with length of time of exposure to heat (from 60.88 ± 6.44 to

103.52 ± 2.61 mmol m^{-2} s^{-1} day 6 to 11, Fig. 6C and Supplementary Fig. S5C). During the peak of the heat wave (days 6 to 11), g_s of HW plants dropped significantly from morning to afternoon (77% decrease morning-afternoon, $P < 0.001$, average of the period)); this trend was not observed in CW plants (day period x temperature $P < 0.0001$, Fig. 5C).

Morning g_s decreased significantly in HW plants from days 11 to 12, but increased in the afternoon (Supplementary Fig. S6C. No changes were observed in HD plants when temperatures returned to normal conditions.

P_n, g_s and E_l in dry plants decreased with increasing VPD_{la} (Fig. 6a, c, e). Under well-watered conditions, P_n and g_s did not follow a clear pattern in response to increasing VPD_{la} until 4 kPa; at this point, both declined with increasing VPD_{la} (Fig. 6b, f). E_l in well-watered plants increased with increasing VPD_{la} up to 4 kPa and declined thereafter (Fig. 6d), whereas it remained stable and very low in dry plants (Fig. 6c).

Chlorophyll fluorescence

The combined heat and drought stress did not affect the maximum efficiency of PSII (Fv/Fm, Table 1) on day 6 (first day with $T_{max} = 40$ °C), when a rather high variability within each group of plants was observed. On day 11 (last day with $T_{max} = 40$ °C), however, Fv/Fm decreased significantly in heated plants compared to control temperature plants (8% decrease, Table 1). Such an effect was also recorded on day 12 (recovery day with $T_{max} = 30$ °C). Most of the absorbed radiative energy (ca. 60%), irrespective of irrigation and temperature conditions, was dissipated as heat through the regulated energy dissipation pathway (Y(NPQ)), while a lower amount was effectively used for photochemistry (φPSII) and non-regulated heat dissipation processes (Y(NO), Table 1). On day 6 (first day), the energy dissipation was unaffected by heat or irrigation level (Table 1). However, on day 11 (last day of the heat wave), the energy partitioning in the plants under high temperature was affected both by heat and water stress (Table 1). The effective photochemical quantum yield of PSII, φPSII, decreased significantly under heat stress in both HW and HD plants (Table 1), while the regulated energy dissipation, Y(NPQ) increased only in HW but not in HD plants (Table 1). Conversely, the non-regulated heat dissipation, Y(NO), increased in plants under combined stress (HD) compared to those under single stress only (HW and CD) (Table 1, Last day). Decreasing the temperature in the heat chamber (recovery) caused a shift of the energy partitioning in HW plants: φPSII increased, while Y(NPQ)

Table 1 Maximum efficiency of PSII (Fv/Fm), effective photochemical quantum yield of PSII, Y(II), quantum yield of light-dependent non-photochemical fluorescence quenching, Y(NPQ), and quantum yield of light-independent non-photochemical fluorescence quenching, Y(NO) in control well-watered (CW), control drought (CD), heat well-watered (HW) and heat drought plants (HD) measured during the afternoon (3 pm – 5 pm) on day 6 (first day with $T_{max} = 40$ °C), day 11 (last day with $T_{max} = 40$ °C) and day 12 (recovery of temperature with $T_{max} = 30$ °C)

Treatment	Fv/Fm			Y(II)			Y(NPQ)			Y(NO)		
	First day	Last day	Recovery	First day	Last day	Recovery	First day	Last day	Recovery	First day	Last day	Recovery
CW	0.68+0.03	0.73+0.01	0.75+0.00 a	0.21+0.02	0.21+0.02	0.20+0.02	0.58+0.01	0.59+0.01	0.61+0.02 a	0.21+0.01	0.20+0.01	0.19+0.01
CD	0.74+0.02	0.74+0.01	0.73+0.02	0.2+0.01	0.20+0.01	0.20+0.01	0.58+0.02	0.61+0.02	0.58+0.01	0.22+0.00	0.19+0.00 b	0.21+0.01
HW	0.65+0.04	0.67+0.03	0.71+0.01	0.19+0.02	0.17+0.00	0.22+0.01 A	0.59+0.02	0.63+0.01 A	0.57+0.01 Bb	0.22+0.01	0.20+0.02 B	0.21+0.01
HD	0.67+0.02	0.67+0.02	0.69+0.02	0.17+0.01	0.17+0.01	0.17+0.00 B	0.61+0.01	0.59+0.01 B	0.60+0.01 A	0.23+0.01	0.24+0.01 Aa	0.23+0.01
Irrigation	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Temperature	ns	**	**	ns	**	**	ns	ns	ns	ns	ns	**
Interaction	ns	ns	ns	ns	*	*	ns	*	*	ns	*	ns

Values are means ± SE. "ns" indicates non-significant effect of the treatments within each day; significant effects of the irrigation and temperature treatments and its interaction are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$). Values are means ± SE. "ns" indicates non-significant effect of the treatments and "**" indicates a significant effect of temperature at $P < 0.01$. Lower- and upper-case letters indicate significant differences between temperature levels and irrigation treatments, respectively

Table 2 Leaf area and shoot length (total per plant and in secondary shoots) and chlorophyll content at the end of the experiment (day 13) in control well-watered (CW), control drought (CD), heat well-watered (HW) and heat drought plants (HD). Values are reported in means $\pm$ SE

Treatment	Leaf area (m^2 plant^{-1})		Lateral shoot length (cm plant^{-1})	Chlorophyll (μg cm^{-2})
	Total	Lateral shoot		
CW	1.37 ± 0.03	0.59 ± 0.04	403.25 ± 46.09	19.58 ± 1.64
CD	1.07 ± 0.07	0.41 ± 0.06	236.33 ± 52.8	15.13 ± 1.74
HW	1.20 ± 0.06	0.54 ± 0.02	323.0 ± 33.53	18.82 ± 0.83
HD	1.10 ± 0.05	0.38 ± 0.04	169.16 ± 42.63	16.56 ± 1.1
Irrigation	***	***	***	*
Temperature	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

"ns" indicates non-significant effect of the treatments; within each day, significant effects of the irrigation and temperature treatments and its interaction are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$)

decreased (Table 1). Both drought and heat stress caused a slight, but significant, increase of the non-regulated energy dissipation Y(NO) (Table 1).

Biometric measurements and chlorophyll concentration

Leaf chlorophyll concentration was lower in dry vines than in irrigated ones (Table 2), while it was unaffected by heat. Similarly, the growth of lateral shoots, developed during the period inside the chambers, decreased significantly in dry plants as compared to well-watered ones but was unaffected by the heat treatment. No significant interactions between irrigation and temperature treatments were found in the biometric parameters (Table 2).

Discussion

In this experiment, we simulated a heat wave with a peak period of 6 days (T_{max} of 40 °C and T_{min} of 24 °C; 30 °C and 17 °C under control conditions) (Supplementary Fig. S1). To disentangle the effects of temperature from soil water availability, the heat wave period started when two contrasting soil water availabilities were already present (Fig. 1). During the peak of the heat wave (days 6 to 11), the maximum daily air VPD reached ca. 5 kPa (Supplementary Fig. S1) and soil water potential values of dry plants ranged from -400 to -800 kPa (0.12 to 0.10 m^3 m^{-3} volumetric soil water content, Supplementary Fig. S3), which

is below the critical threshold at which evapotranspiration starts to decrease due to the soil moisture deficit (-700 MPa and 0.165 m^3 m^{-3}, estimated for different vegetation types across Europe) (Fu et al. 2022).

Stomatal responses to soil water availability played a central role in plant responses to the heat wave, with two clearly distinct behaviors under dry and well-watered conditions. In general, Pn, gs and E_l significantly responded to the heat wave when plants were watered, while the response was often minor when vines suffered from water stress. Even before the onset of the heat wave, on day 3, when soil water potential reached -250 kPa (Fig. 1), g_s, E_l, and P_n were severely reduced by the low soil water availability in dry plants (71, 70 and 70% reduction respectively from CW plants on daily average, Fig. 5), indicating diffusional limitations of P_n due to the stomatal closure in response to the water deficit (Flexas et al. 2000; Flexas and Medrano 2002). The fact that the drought treatment did not affect Fv/Fm (Table 1) confirms that the decrease in P_n was mainly due to stomatal closure and not to impairment in the PSII under low water availability. Wenter et al. (2022) also found a severe drop in stomatal conductance in grapevine leaves at similar values of soil water potential. This mechanism has likely prevented stem water potential from reaching values below -1.5 MPa during our experiment, which can cause severe cavitation in grapevines (Lovisolo et al. 2008; Charrier et al. 2018). A strong stomatal control, responsible for maintaining relatively constant values of stem water potential despite the increasing limitation of

water availability in the soil (Fig. 2), is characteristic of specific grapevine cultivars, such as Sauvignon blanc (Gutiérrez-Gamboa et al. 2019; Gambetta et al. 2020). The minimum values of Ψ_{stem} recorded in dry plants until day 10 ranged from -0.85 to -1.1 MPa (Fig. 2) which, if considered alone, would indicate only mild water stress according to Romero et al. (2022). However, upon considering the gas exchange and growth data (lateral shoot length and leaf area, Table 2), it becomes evident that dry plants were actually subjected to a rather severe drought stress.

When vines experiencing drought conditions were exposed to the heat wave, a further slight decrease in g_s and P_n was recorded exclusively during the rise of the heat wave (days 4 to 6, Fig. 5A, C), while E_l did not decrease further during the heat wave (Fig. 5B). The heat conditions initially caused a stomal closure (Fig. 6), which in turn triggered an increase in Ψ_{stem} of HD plants with respect to CD (day 5, Fig. 2), followed by a progressive decline in Ψ_{stem} from days 5 to 10, when soil water potential in dry plants reached increasingly negative values.

When soil water potential in dry plants was between -400 and -800 kPa and VPD reached the maximum of the experiment (peak of the heat wave), there were no or very small further effects of heat on g_s and consequently on P_n and E. This is in line with a similar study reporting no effects of heat stress on g_s when heat (45 °C for 48 h) was combined with drought in grapevine cultivar Cabernet sauvignon (Tan et al. 2023). However, other experiments exploring the effects of combined stresses on other grapevine cultivars found lower g_s values in combined stress as compared to single drought (Edwards et al. 2011; Lehr et al. 2021). Comparing different studies is however often challenging due to significant discrepancies in experimental designs and the genotypes (cultivars and rootstocks) employed. For instance, in the study by Edwards et al. (2011), the duration of the heat period was shorter (40 °C maximum temperature for only two days), whereas in Lehr et al. (2021), the duration was similar (seven days), but with a lower maximum temperature (39 °C) compared to the one employed in the current experiment (Supplementary Fig. S1). Furthermore, substantial differences among the experiments were observed in terms of pot volume. In our experiment, the pot size was significantly larger (50 L) compared to the others: 3 L (Galat-Giorgi et al. 2019), 5 L (Edwards et al. 2011; Tan et al. 2023), and 15 L (Lehr et al. 2021). The higher exploitable soil volume could likely have influenced overall root development, consequently affecting the timing and intensity of plant responses to the increasing water limitation and temperatures.

Under well-watered conditions, HW plants reacted to the heat wave by almost doubling the daily E_p as compared to CW plants (Fig. 3), which lowered leaf temperature in HW plants by 3.5 °C in comparison to HD plants (average during the peak of the heat wave, Supplementary Fig. S4). The decrease in temperature in CW plants compared to CD plants was only 1.72 °C (average during the peak of the heat wave, Supplementary Fig. S4). The temperature effect on the transpiration of HW plants was also confirmed by its severe drop after the end of the heat wave (Fig. 3). The increase in E_p and leaf gas exchange parameters in HW vines occurred during the morning, when the temperature was approximately 35 °C (Fig. 4 and Fig. 5); such effect was not observed in the afternoon, when the peak in T_{max} was reached (40 °C). Given that the changes in plant water availability from the morning to the afternoon were minimal (Supplementary Fig. S7 and S8), these differences are most likely due to a combined effect of air temperature and VPD on stomatal regulation and hence on plant transpiration. In fact, all gas exchange parameters severely decreased from the morning to the afternoon in HW plants (78, 57 and 78% decrease from morning to afternoon in P_n, E_l and g_s on average during the peak of the heat wave, Fig. 5). We cannot rule out the possibility that a fraction of total leaf transpiration of HW plants resulted from cuticular transpiration as an additional mechanism to increase heat tolerance (Riederer and Schreiber 2001) because despite the strong g_s reduction in HW plants in comparison to CW plants in the afternoon (50% average reduction in HW plants compared to CW plants on days 6 to 11, Fig. 5C), the transpiration at leaf level was in fact similar in HW and CW plants (afternoon, Fig. 5B).

The maximum efficiency of PSII, indicated by the Fv/Fm ratio, significantly declined under exposure to heat stress, which suggests some damage in the thylakoid membranes, and consequently in PSII. This is consistent with the results of Wang et al. (2009) and Zha et al. (2019) after a short (2–6 h) exposure of several genotypes of *V. labrusca* and grapevine hybrids to 45–47 °C. Our results also agree with those of Kadir et al. (2007), who recorded a decline in Fv/

Fm in grapevines after 12 days of exposure to 40 °C, but disagree with the findings of Galat-Giorgi et al. (2019), who found no change in Fv/Fm when subjecting grapevines of the cv. Malbec to a combined heat and water stress. This disagreement could be partially explained by the higher ambient light intensity used in our experiment (1500 vs 800 µmol m^{-2} s^{-1}), and thus to a higher excitation energy, which might have exacerbated the effects of heat and drought stress. The Fv/Fm ratio, however, never decreased below 0.66, suggesting no irreversible damage in the photosystem (Palliotti et al. 2009).

The fate of the absorbed radiation energy in PSII differed under single and combined stress after a prolonged stress exposure (Table 1). Although generous water availability did not improve the energy use for photochemistry under heat stress, it altered the direction of the absorbed energy between regulated and non-regulated energy dissipation pathways (Y(NPQ) and Y(NO), respectively). Watered plants in the heat chambers were, in fact, able to protect PSII by dissipating more energy through regulated non-photochemical processes, Y(NPQ), while those under combined heat and water stress (HD) increased the proportion of energy lost by harmful non-regulated dissipative pathways, Y(NO). We hypothesize that this differential behavior is linked to the fact that well-watered plants in the heated chambers maintained higher transpiration rates than those subjected to both types of stresses (Figs. 3 and 4), which resulted in lower leaf temperatures in well-watered plants (Supplementary Fig. S4). The shift in the dissipation energy partitioning under heat stress occurs very rapidly after the onset of the heat stress, as reported by Wang et al. (2009), who recorded a downregulation of energy use for photochemistry φPSII and higher energy dissipation as harmless heat (Y(NPQ)) after only 1 h of exposure of grapevines to 47 °C. After only 2 h at such high temperatures, however, Y(NPQ) also decreased and Y(NO) increased. High Y(NO) values are associated with a longer lifetime of energy excitation that increases the probability of reactive active oxygen species formation (Samson et al. 2019). After decreasing the temperature, water availability showed a notable positive effect on energy partitioning. HW plants were able to drive more energy to photochemistry and less energy to regulated

energy dissipation compared to HD plants, in agreement with the recovery observed in CO_2 assimilation on day 12. However, well-watered plants did not recover their Fv/Fm ratio at the end of the simulated heat wave after decreasing the temperature, as indicated by the Fv/Fm ratio on day 12. These results are in line with Kadir et al. (2007), who reported the first recovery of Fv/Fm from heat stress after 3 days of decreasing the temperature, while a complete recovery was recorded after 12 days.

When plants are subjected to multiple environmental stresses, such as high temperature and drought, photoinhibition is likely to occur. Under drought conditions, CO_2 fixation is impaired by the limited availability of CO_2 due to reduced stomatal conductance, and the energy used by the Calvin cycle decreases, which results in an excess of excitation energy (Chaves et al. 2009). When heat stress adds up, the need for a protection mechanism to dissipate this excess energy increases. The first pathway to dissipate the excess energy is regulated thermal dissipation that competes with the photochemical energy pathway. In our study, regulated non-photochemical heat dissipation increased, while the photochemical yield decreased under heat stress.

When the above-mentioned mechanism is restricted or damaged due to severe stress, other electron dissipation mechanisms (such as the Mehler reaction) participate as an alternative electron sink to dissipate the excess energy, which results in an overproduction of ROS and consequently in damages to PSII reaction centers (Savitch et al. 2009). The production of ROS caused by stress exposure increases the need for antioxidants such as L-Ascorbic acid and Glutathione, which play an important role in eliminating ROS through the ascorbate–glutathione cycle. However, under combined heat and drought stress, the production of these antioxidants tends to decrease, indicating a difficulty in maintaining the cellular redox state (Carvalho et al. 2015), which might explain the increased values of Y(NO) found in our study as well as in that by Wang et al. (2009), as a consequence of heat stress.

During the recovery phase, a reorientation of energy partitioning in PSII occurred, compared to that during the heat wave. HW plants, and not HD plants, resumed photochemistry activity and decreased energy partitioning through regulated non-photochemical dissipation.

Conclusions

Taken together, our results indicate that an adequate soil water availability allowed grapevines to react to a heat wave by enhancing leaf photosynthesis and transpiration in the morning and maintaining values like irrigated plants at control temperatures during the afternoon. When grapevines were already exposed to drought, the effects of the heat wave were negligeable, with drought plants showing similar leaf photosynthesis and transpiration at control and elevated temperatures. The combined heat and drought stress, however, increased the proportion of energy lost by the leaves through harmful non-regulated dissipative pathways, a risk that was, at least in part, avoided by well-watered plants under high temperatures probably because of the leaf cooling effect driven by transpiration. The significant increase of grapevine transpiration will likely affect the irrigation water needs of grapevines during heat waves which could pose a threat to irrigation water availability in areas with limited water resources and may thus result in conflicts between different water users.

Data availability   Data is available upon request.

Declarations

Competing Interests   No conflict of interest declared.

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