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Marino, Giulia
Scalisi, Alessio
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Detecting Mild Water Stress in Olive with Multiple Plant-Based Continuous Sensors

Giulia Marino [1,2,*], **Alessio Scalisi** [2,3], **Paula Guzmán-Delgado** [1], **Tiziano Caruso** [2], **Francesco Paolo Marra** [2] **and Riccardo Lo Bianco** [2,*]

[1] Department of Plant Sciences, University of California, Davis, CA 95616, USA; pguzmandelgado@ucdavis.edu
[2] Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, 90133 Palermo, Italy; alessio.scalisi@agriculture.vic.gov.au (A.S.); tiziano.caruso@unipa.it (T.C.); francescopaolo.marra@unipa.it (F.P.M.)
[3] Agriculture Victoria, Department of Jobs, Precincts and Regions, Tatura, VIC 3616, Australia
[*] Correspondence: giumarino@ucdavis.edu (G.M.); riccardo.lobianco@unipa.it (R.L.B.)

Abstract: A comprehensive characterization of water stress is needed for the development of automated irrigation protocols aiming to increase olive orchard environmental and economical sustainability. The main aim of this study is to determine whether a combination of continuous leaf turgor, fruit growth, and sap flow responses improves the detection of mild water stress in two olive cultivars characterized by different responses to water stress. The sensitivity of the tested indicators to mild stress depended on the main mechanisms that each cultivar uses to cope with water deficit. One cultivar showed pronounced day to day changes in leaf turgor and fruit relative growth rate in response to water withholding. The other cultivar reduced daily sap flows and showed a pronounced tendency to reach very low values of leaf turgor. Based on these responses, the sensitivity of the selected indicators is discussed in relation to drought response mechanisms, such as stomatal closure, osmotic adjustment, and tissue elasticity. The analysis of the daily dynamics of the monitored parameters highlights the limitation of using non-continuous measurements in drought stress studies, suggesting that the time of the day when data is collected has a great influence on the results and consequent interpretations, particularly when different genotypes are compared. Overall, the results highlight the need to tailor plant-based water management protocols on genotype-specific physiological responses to water deficit and encourage the use of combinations of plant-based continuously monitoring sensors to establish a solid base for irrigation management.

Keywords: fruit gauge; *Olea europaea* L.; precise water management; stem water potential; turgor pressure; water relations

1. Introduction

Climate change and water scarcity, along with an intensification of agricultural systems, are urging the reduction of irrigation volumes applied in agricultural systems. Intensive olive orchards (300–600 tree ha^{-1}) are more productive per surface unit with respect to traditional systems (less than 300 tree ha^{-1}), but they are more sensitive to water deficit. Hence, plant-based irrigation management is a key strategy to maintain economical profitability and environmental sustainability in intensive systems [1].

Among the different indicators of plant water status, stem water potential (Ψ_{stem}) is currently the most widely studied and used. Thus, crop-specific protocols are available in the literature to manage irrigation in fruit tree systems using midday Ψ_{stem}—the Ψ_{stem} at solar noon—as an indicator of water stress [2,3]. In olive, two recent studies developed in two different environments (Sicily and Argentina) and with multiple cultivars (Arbequina, Nocellara del Belice, and Olive di Mandanici) suggested −2.0 MPa as a midday Ψ_{stem} threshold for mild water stress since a clear and gradual decrease in stomatal conductance

and net photosynthesis occurred at Ψ_{stem} below this value [4,5]. However, when Ψ_{stem} was above -2 MPa, the high variability of gas exchange was not reflected in Ψ_{stem} changes. This is probably associated with the variable near-isohydric behavior of some olive cultivars that influence the degree of Ψ_{stem} changes at low water stress levels [4,6]. Thus, alternative indicators to midday Ψ_{stem} are needed to improve the precision of water stress detection at $\Psi_{stem} \geq -2$ MPa and maximize water productivity in olive orchards.

In recent years, thanks to the rapid advancement of technologies, various plant-based sensors have been tested and proposed for automated water management in orchard systems. Most of these sensors do not measure plant water status directly but they monitor specific physiological processes that correlate in different ways and degrees with plant water status. Since tree physiological responses are mediated by several factors, including tree phenological stage, environmental conditions, or genotype-specific traits, the development of simplified and uniform water management protocols using these sensors is complex [7,8]. As an example, the use of the leaf patch clamp pressure (LPCP) probe [9] could be very promising for irrigation scheduling in 'Arbequina' olive orchards [10,11]. This sensor measures the variability in the output pressure (P_p, kPa) between two magnets clamped to a leaf. When trees are not water-stressed, P_p is a good proxy of the attenuation of turgor pressure (P_c), with P_p and P_c being inversely related. For instance, when leaf turgor pressure decreases in response to daytime stomatal opening, P_p gradually increases while when stomata close at night and turgor pressure increases, P_p gradually decreases (phase 1). However, when trees enter water deficit, a clear decrease of P_p values associated with the accumulation of excess air in leaf tissues occurs, resulting in a semi-inversion of the P_p diurnal trend, with a depression in the middle of the day (around solar noon, phase 2) [12]. More severe stress will cause a complete inversion of the P_p curve, with high peak in the night hours and lower values in the day hours (phase 3). An example of the above mentioned phases of inversion is reported in Figure 1.
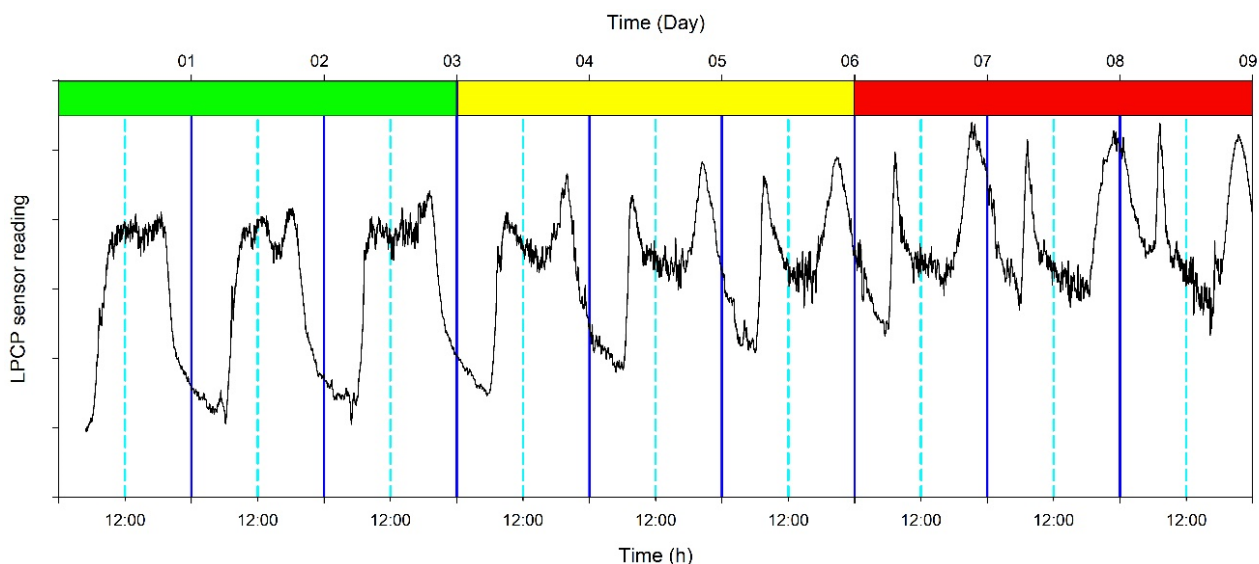

Figure 1. Example of the stress phases based on the inversion of the leaf patch clamp probe (LPCP) output pressure (P_p) reading, with the green area showing the phase 1 or no stress (no inversion of the P_p curve, P_p equal to the inverse of turgor pressure); the yellow area indicating phase 2 or stress onset (half-inversion of the P_p curves or semi-inversion) and the red area showing phase 3 or high stress (full inversion of the P_p curve). Dark blue vertical lines indicate midnight and light blue dashed vertical lines indicate midday.

This change in the shape of the diurnal P_p curve was associated with a midday $\Psi_{stem} < -1.2$ MPa for the cultivar Arbequina grown in Southern Spain [10,11]. However, when this sensor was tested on different olive cultivars and environments, an evident change in the shape of the P_p curve was only clearly observed at midday $\Psi_{stem} < -2.5$ MPa [13].

'Arbequina' is an olive cultivar selected in temperate regions and has shown a lower capability to adapt to drought, and a higher bulk elastic modulus (ε) (i.e., a higher tissue rigidity) than other cultivars [14]. A high ε is generally associated with a low capability of the cells to maintain turgor, which probably influenced the high sensitivity of the P_p daily curve to water stress in this cultivar.

Some recent studies suggested combining the use of different plant-based sensors to improve water stress detection in olive. Scalisi et al. [7] reported that combining leaf and fruit sensing improves water stress detection in two olive cultivar. Girón et al. [15] investigated physiological responses of olive fruits under different levels of water deficit (with midday $\Psi_{stem} < -2.5$ MPa) and found that, at moderate water stress, fruits are weaker water sinks than leaves, as opposed to what is observed in other species [16,17]. Yet, when the severity and duration of water stress increases, fruit sink activity may become stronger than leaf sink activity for water. Rodríguez-Domínguez et al. [18] combined stem sap flow and leaf turgor pressure measurements and demonstrated that different indicators should be used depending on the intensity of stress, with sap flow being a potential solution to detect severe water stress when the P_p curves are inverted. All these studies highlight the complexity of the detection of water stress in olive, particularly for light to mild water stress conditions and when different cultivars are considered. Therefore, a more comprehensive characterization of water stress onset is needed for the development of automated irrigation protocols aiming to increase olive orchard environmental and economic sustainability.

The main aim of this study was to determine whether a combination of continuous leaf turgor, fruit growth and sap flow responses improves the detection of mild water stress in olive—i.e., when trees approach a midday Ψ_{stem} threshold of -2.5 MPa, before the inversion of the P_p curves. In addition, two Sicilian olive cultivars (Olivo di Mandanici and Nocellara del Belice) were investigated to assess if the combined use of these physiological indicators could provide useful information, regardless of the cultivar-specific response to water deficit. In particular, 'Olivo di Mandanici' (OM) showed a slower decrease in Ψ_{stem} and a lower tendency to show semi-inversion or inversion of the P_p curves in response to water withholding with respect to 'Nocellara del Belice' (NB) [7,13,19], resulting in lower irrigation needs.

The first hypothesis was that a combination of physiological parameters provides better indications of mild water stress than individual indices. The second hypothesis was that physiological responses to stress may differ in cultivars with different sensitivity to drought, thus requiring the use of alternative indicators to assess mild water stress.

2. Materials and Methods

2.1. Orchard Characteristics

The experiment was carried out in an intensive olive orchard located near Sciacca (37′32″ N, and 13′02″ E, 150 m a.s.l.) in Southwestern Sicily (Italy) during August and September 2015.

The soil is a sandy clay loam (60% sand, 18% silt, 22% clay), with pH 7.7 and active carbonates lower than 5%. The orchard was planted in 2012 with seven different Sicilian genotypes previously selected by the Department of Agricultural, Food and Forest Sciences at the University of Palermo. The density was 666 trees ha^{-1}, with a spacing of 5 m between rows and 3 m on North-South oriented rows. Trees were trained to free palmette with a single trunk and three scaffolds: the first at 0.5–0.6 m from the ground, the second at 1.30–1.40 m, and the third at 2.10–2.20 m. All trees received the same conventional cultural cares from planting until the beginning of the current experiment.

2.2. Environmental Conditions

The climate is Mediterranean, with an average yearly precipitation of 516.2 mm and an average monthly temperature of 18.2 °C (period 1965–1994, meteorological station of Sciacca, Servizio Informativo Agrometerologico Siciliano). Data of average daily tempera-

ture and average daily relative humidity used to calculate vapor pressure deficit (VPD), as well as total daily precipitation were obtained from the meteorological station of Sciacca.

At the beginning of August, the weather was particularly hot and dry, with VPD values as high as 4 kPa (Figure 2). A rain event on 8 August, decreased the VPD below 2 kPa for about a week. VPD showed a constant pattern in the second half of August, with daily minimum values of ~0.5 and maximum values of ~2.5 kPa. At the beginning of September, a heavy rain event (>50 mm) caused a marked decrease in the daily maximum VPD values to ~1 kPa. A steep increase of VPD was then observed for the 5 days between 16 and 20 September.
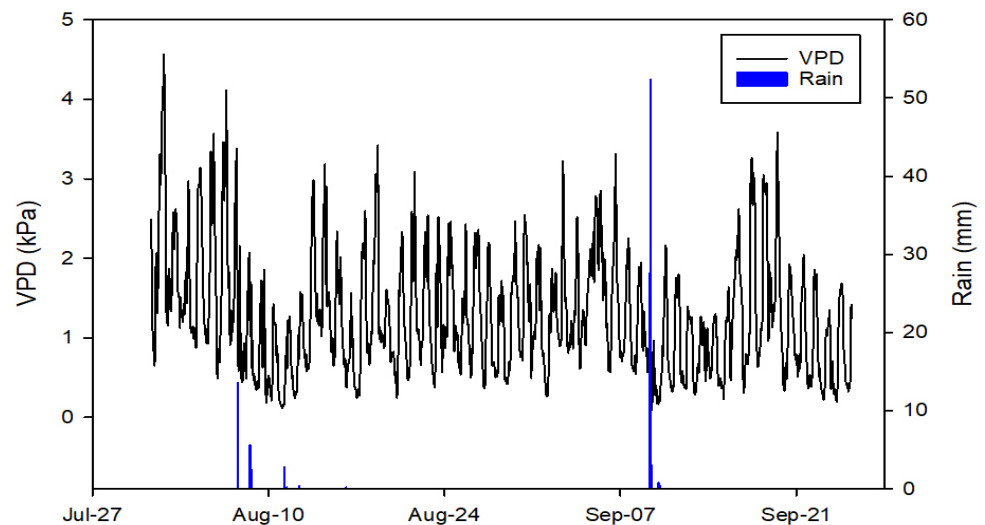

Figure 2. Seasonal trend of vapor pressure deficit (VPD, hourly mean) and total daily rain (from hourly values) from 1 August to 26 September 2015, obtained from data of the meteorological station in Sciacca, Sicily, Italy.

2.3. Irrigation Management and Experimental Design

Irrigation water was supplied by two self-compensating in-line drippers per plant delivering 16 L h^{-1}. In mid-July, when pit hardening usually occurs in the experimental area, trees were not irrigated until their midday Ψ_{stem} decreased below -2.5 MPa. The main aim was to keep the trees under a mild level of stress (midday Ψ_{stem} between -2 and -2.5 MPa) based on the results of Marino et al. [4]. In September, when the oil accumulation rate is generally high, irrigation was applied to avoid water stress and keep the Ψ_{stem} above -2.0 MPa. The experimental design was composed of three blocks per cultivar made of three adjacent trees each and randomized within the row. The two cultivars were displayed in two separate rows. Measurements were performed in the central tree of each block. One sensor of each type was installed in each selected tree, for a total of 18 sensors (three sensor-types per three trees per two cultivars). The sampling procedure and number of measurements corresponded to the standards for these settings and type of investigation [7,19] and fully support the strength of the presented outputs.

2.4. Measurements

Plant water status was monitored weekly on nine trees per cultivar measuring midday Ψ_{stem}. One current-year west-oriented shoot with five or six pairs of fully expanded leaves per selected tree was covered with plastic wraps and aluminum reflective foils at least 1 h before measurements to reduce leaf transpiration and equilibrate branch water potential with tree water potential [20] Shoots were then detached and Ψ_{stem} was measured using a pressure chamber (PMS Instrument Co., Model 600D, Corvallis, OR, USA).

Starting from 1 August, LPCP probes (YARA ZIM Plant Technology GmbH, Hennigsdorf, Germany), sap flow sensors (Ecomatik, UP-GmbH, Dachau, Germany), and fruit

gauges based on linear variable displacement transducer (LVDT) sensors were installed on the selected trees to monitor relative changes in leaf turgor pressure, sap flow density (Q, mL cm^{-2} min^{-1}), and micrometric fruit diameter variations, respectively.

The LPCP probes were installed following reported methodology [9,21] on fully expanded leaves of west-oriented, current-year shoots, similar to the ones used for Ψ_{stem}. The output signals of the LPCP probes (P_p, kPa) were standardized using z-scores [i.e., z = (x − mean)/standard deviation] to allow comparisons among leaves with different initial turgor pressure when sensors were mounted. The inversion of the P_p daily curve was used to characterize the following three stress phases based on [12]: phase 1 or no stress, the leaf is in a turgescent state characterized by an inverse relationship between P_p and P_c, and midday Ψ_{stem} is above ~−2.0/2.5 MPa; phase 2 or mild stress, characterized by semi-inverted P_p curves and midday Ψ_{stem} below ~−2.0/2.5 MPa but above ~−3.0/−3.5 MPa; phase 3 or severe stress, the leaf is significantly dehydrated (unfavorable ratio of air to water in the leaf tissue) and midday Ψ_{stem} is lower than ~−3.0/−3.5 MPa (in this state, the P_p daily curve is inverted compared to phase 1, i.e., P_p reaches minimum values around solar noon and maximum values during the night). Reported midday Ψ_{stem} values for each phase were developed in a previous study performed in the same study orchard [13].

Granier-type heat dissipation probes [22] were used to measure sap fluxes. The sap flow probes were placed on the west side of the trunk at 50 cm from the ground and covered with aluminum foil to avoid the effect of direct sunlight on sensor temperature readings. The temperature signals from the probes were recorded at 30-min intervals using a CH6 data logger (GMR Strumenti Sas, Scandicci, Florence, Italy).

The sap flow density (Q, mL cm^{-2} min^{-1}) was calculated from the temperature difference between the two needles (ΔT) and the maximum value of ΔT ($\Delta Tmax$) at night as follows [22]:

$$Q = 0.714 \times [(\Delta Tmax − \Delta T)/\Delta T]$$

Fruit gauges were installed on fruits located in the same main branches where the LPCP probes were installed, as close as possible to the leaf sensors, and connected to CR1000 data loggers (Campbell Scientific Inc., Logan, UT, USA) set to record micrometric variations (mV) at 15-min intervals. Data in mV were subsequently converted in μm starting from an initial fruit diameter measured with a digital caliper and considering that a change in 1 mV was equivalent to 3.3 μm. Fruit diameter data were subsequently smoothed by applying a filter using a 15-point convoluted spline to remove noise [23].

Relative growth rate (RGR, μm mm^{-1} min^{-1}) was calculated using the following equation:

$$RGR = (\ln D2 − \ln D1)/(t2 − t1)$$

where D1 and D2 are fruit diameter at times t1 and t2, respectively.

2.5. Statistical Data Analysis

Exploratory data analysis (EDA) was carried out to assess patterns and anomalies of physiological parameters in time series. Statistical analyses of the data were performed using R program [24]. Analysis of variance was performed with cultivar and time as main factors and Tukey's multiple comparison test was used to separate means. Linear regression analyses between sensor output and Ψ_{stem} were performed and Student's *t*-test was used to compare regression coefficients.

3. Results

3.1. Stem Water Potential

Midday Ψ_{stem} showed only slight differences between the two cultivars throughout the experimental period. In August, midday Ψ_{stem} values were lower than the threshold value of −2.0 MPa, mainly oscillating between −2 and −2.7 MPa (Figure 3). The Ψ_{stem} values were higher in Olivo di Mandanici (OM) than in Nocellara del Belice (NB), both at the beginning and the end of this month. In September, Ψ_{stem} increased to values above

the −2.0 MPa threshold, with NB showing slightly higher values in the second half of the month.
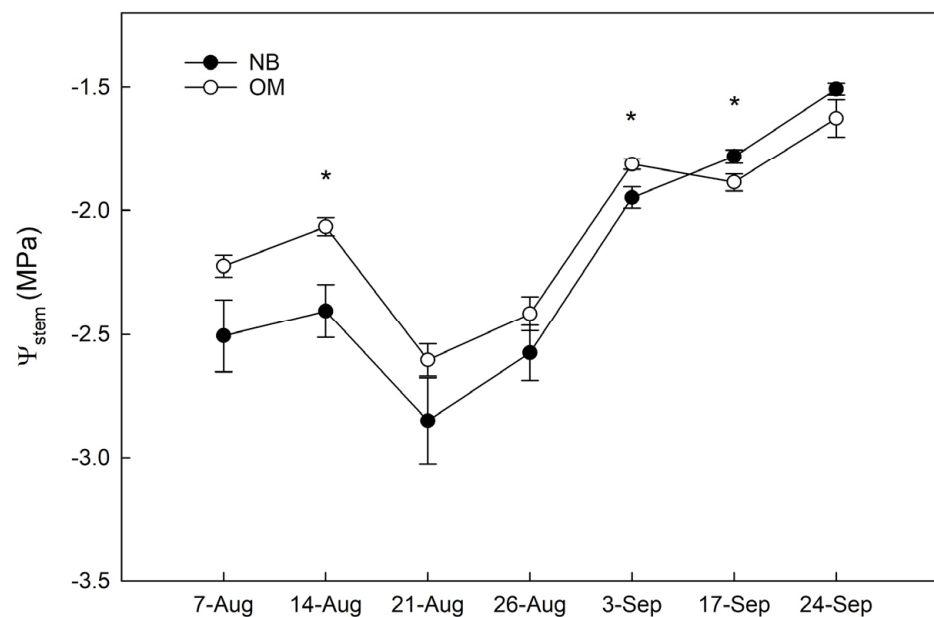

Figure 3. Weekly midday stem water potential (Ψ_{stem}) measured for the olive cultivars Olivo di Mandanici (OM) and Nocellara del Belice (NB). Vertical black bars represent standard errors of the mean (n = 9); asterisks indicate significant differences between means (Tukey's test, $p \leq 0.05$).

3.2. Continuous Trends of Leaf Turgor, Sap Flow and Fruit Relative Growth Rate

A combination of continuous measurements of physiological parameters can provide insight into processes occurring when trees experience an increased water stress, as well as differences associated with cultivar-specific traits. Based on the EDA of the curve daily shape, no stress was detected through almost the entire experimental period, as highlighted by the green circles in Figure 4, indicating a normal, bell-shaped curve for the specific day (phase1 or no stress). Only on five days (27 August for OM, and 7, 19–20, and 27–28 August for NB) did the EDA of the curve daily shape identify some stress level, which was highlighted by yellow and red circles indicating partial or full inversions of the curves during these days (phases 2 and 3 of stress onset and high stress, respectively).

Despite such a low stress detection based on the P_p daily curves, a strong day to day variability in P_p dynamics can be clearly observed between two consecutive irrigation events (Figure 4). This is particularly evident during the second half of August, when daily minimum P_p values (P_{p-min}) increased in both cultivars, with values ranging from about −1, just after the irrigation, to positive values recorded after five to seven days of water withholding (red continuous arrows in Figure 4). The cultivar OM also showed a progressive increase of maximum daily P_p values (P_{p-max}) from ~0.5 up to ~2 between two consecutive irrigation events (red dashed arrows in Figure 4a). In contrast, P_{p-max} was relatively constant in NB, with approximate values of 0.5 to 1.0 (red dashed arrows in Figure 4b).

At the beginning of September, the increased frequency of irrigation re-established relatively constant P_p values for about two weeks, with P_{p-max} between 0 and 1 and P_{p-min} around −1. The cultivar NB had a more stable P_{p-max} and more variable P_{p-min}, while OM had more stable P_{p-min} and more variable P_{p-max}. Starting from mid-September, both cultivars showed a continuous increase of P_{p-min} and P_{p-max} through to the end of the season (Figure 4) most probably in response to the strong and rapid increase in VPD observed in this period (Figure 2).
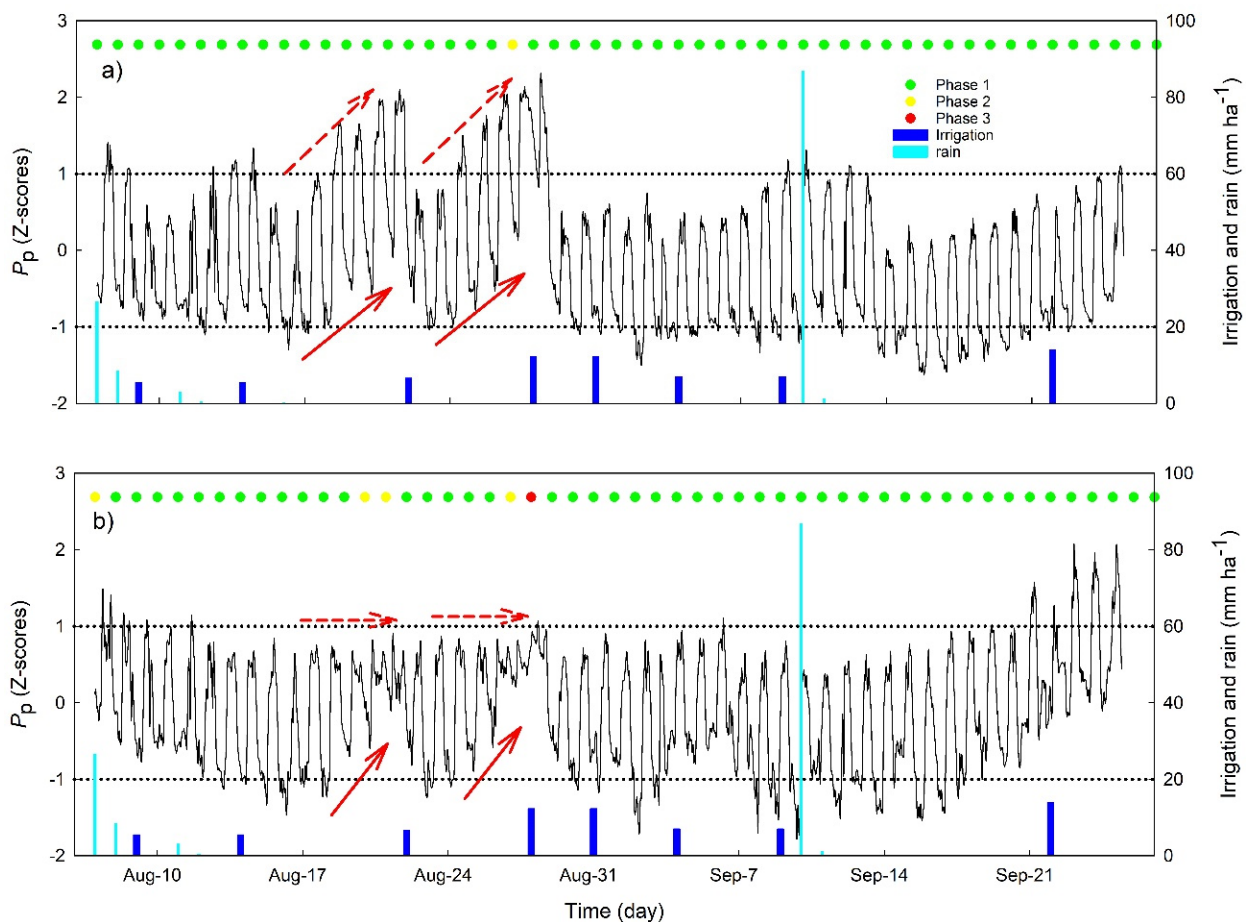
Figure 4. Time course of the hourly standardized leaf patch clamp pressure (LPCP) probe output pressure (P_p) for the olive cultivars Olivo di Mandanici (**a**) and Nocellara del Belice (**b**). Green, yellow and red dots represent daily stress phases based on exploratory data analysis of the diurnal shape of the LPCP probe outputs: phase 1 or no stress (no inversion of the P_p curve); phase 2 or stress onset (half-inversion of the Pp curves), and phase 3 or high stress (full inversion of the P_p curve). The horizontal dotted lines are the z-score of −1 and +1, representing the thresholds within which 68.27% of the results fall. Red arrows highlight the variability of the P_p values between irrigation events discussed in the text.

Fruit RGR dynamics showed clear changes in the period between two irrigation events, with a progressive decrease of the daily minimum RGR value (RGR$_{min}$), from values close to 0, just after the irrigation, to values of −0.3 μm mm^{-1} min^{-1} in OM and 0.15 μm mm^{-1} min^{-1} in NB (Figure 5, red continuous arrows). The cultivar OM also showed an increase of maximum daily RGR values (RGR$_{max}$) between two irrigation events from ~0 up to 0.3–0.4 μm mm^{-1} min^{-1}, while in NB, RGR$_{max}$ was constant between two consecutive irrigation events (red dashed arrows in Figure 5). During all of September, RGR was close to 0 in both cultivars except for the period between 18 and 22 September, when fruits showed larger size fluctuations in response to 10 consecutive days without irrigation.

The dynamics of Q was less clearly affected than the previous parameters by the irrigation events (Figure 6). In August, OM daily Q dynamics showed a very high peak of transpiration rates during the morning (values reaching up to 0.025 L cm^{-2} h^{-1}) but average values for the rest of the day ranged between 0.015 and 0.02 L cm^{-2} h^{-1}. NB did not show a clear morning peak and average values in August were between 0.015 and 0.02 L cm^{-2} h^{-1}. In September, during the 10 days of irrigation withholding, NB Q values increased from 0.018 to 0.024 L cm^{-2} h^{-1}, while OM Q values decreased from 0.021 to 0.015 L cm^{-2} h^{-1}.
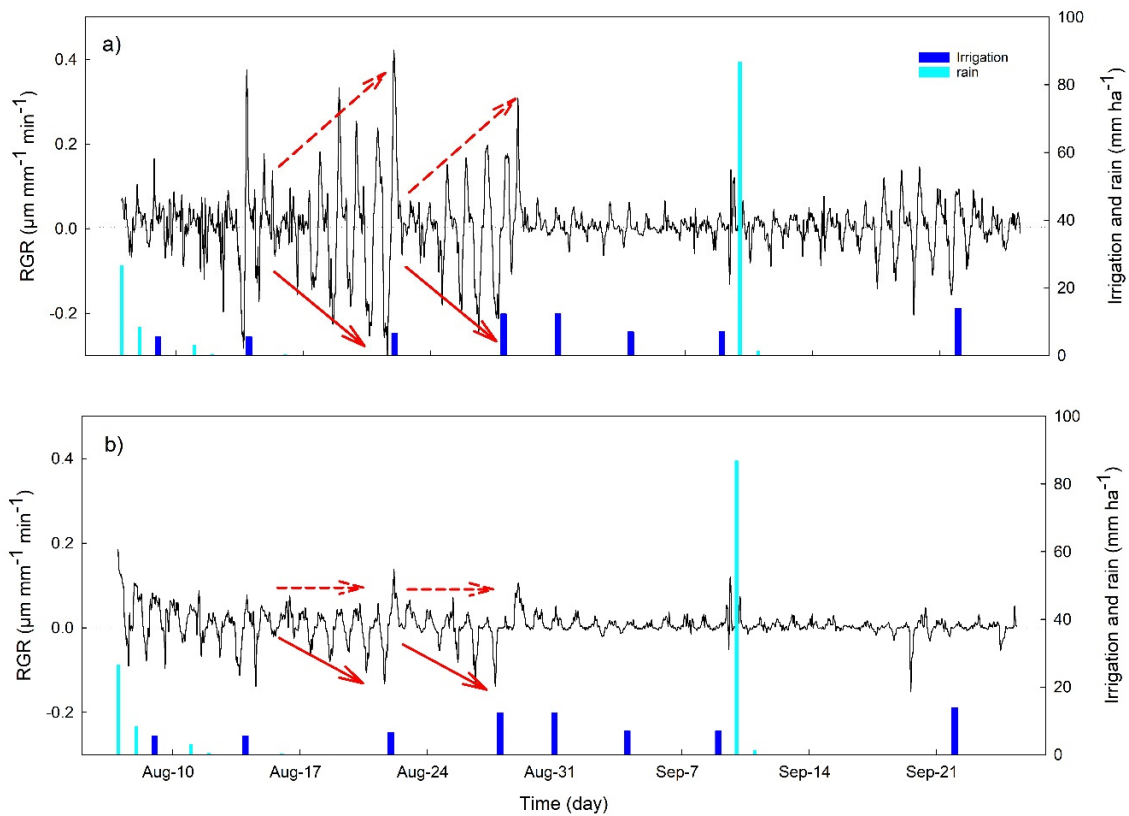
Figure 5. Time course of the hourly fruit relative growth rate (RGR) for the olive cultivars Olivo di Mandanici (**a**) and Nocellara del Belice (**b**). Red arrows indicate the trends of RGR peaks between consecutive irrigation events discussed in the text.
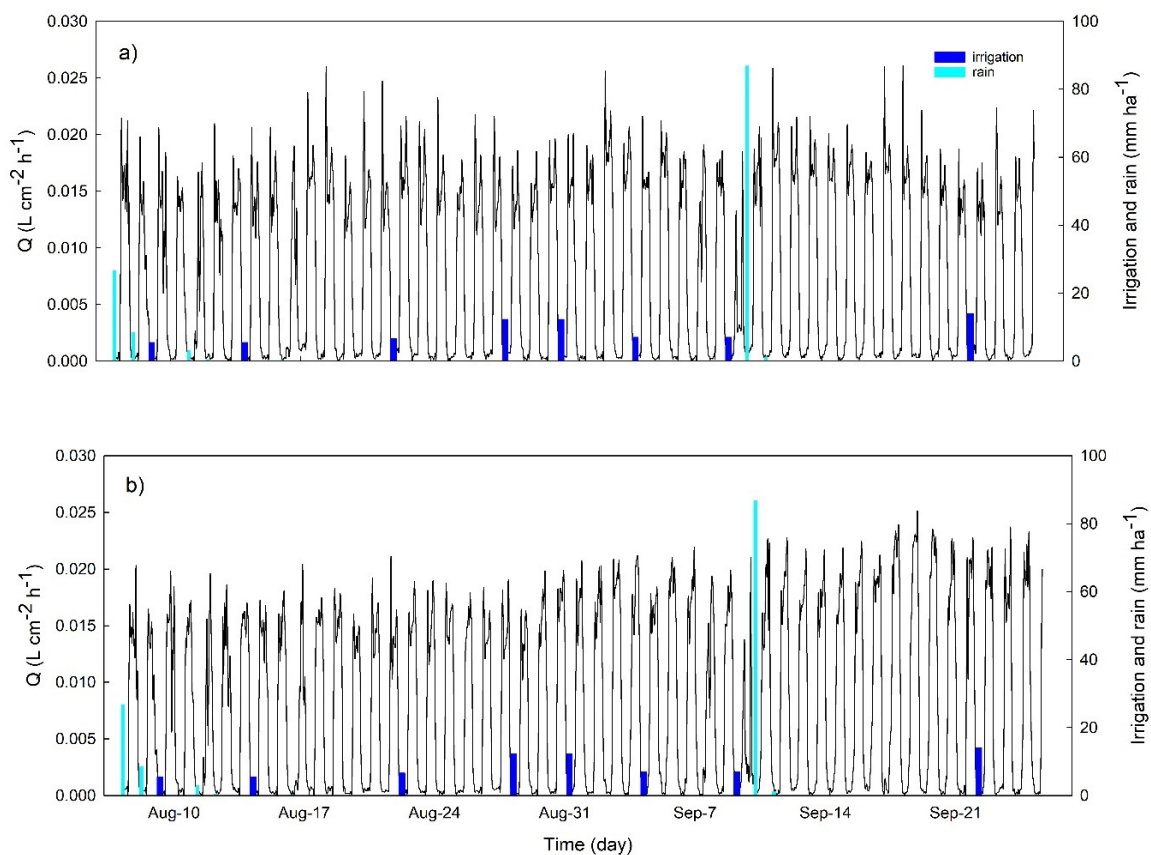

Figure 6. Time course of the hourly sap flow rate (*Q*) for the olive cultivars Olivo di Mandanici (**a**) and Nocellara del Belice (**b**).

3.3. Relationships between Stem Water Potential and Sensor-Derived Physiological Parameters

To gain further insight into the leaf, fruit, and sap dynamics, and evaluate their potential as mild water stress indicators, we analyzed the relationship between midday Ψ_{stem} and the minimum values of P_p ($P_{p\text{-min}}$), the difference between the minimum value and the maximum value of RGR (RGR$_{range}$), and the daily average of Q values (Q_{ave}) (Figure 7). These three parameters or indicators were selected because they showed the highest variability between two consecutive irrigation events, as derived from the hourly data analyses (Figures 3–5). $P_{p\text{-min}}$ showed a significant linear and negative relation with Ψ_{stem} (Figure 7a), with no statistical difference observed between the slopes of the two tested cultivars ($p = 0.3$, Table 1). Similar to $P_{p\text{-min}}$, the RGR$_{range}$ was negatively correlated with Ψ_{stem} (Figure 7b) but the slope was significantly higher in OM than in NB (Table 1, $p = 0.03$). The different response of the two cultivars was also highlighted by the relationship between Q_{ave} and Ψ_{stem}, which was significant, linear, and positive in NB and not significant in OM (Figure 7c), with significant difference between the slopes ($p = 0.001$, Table 1).
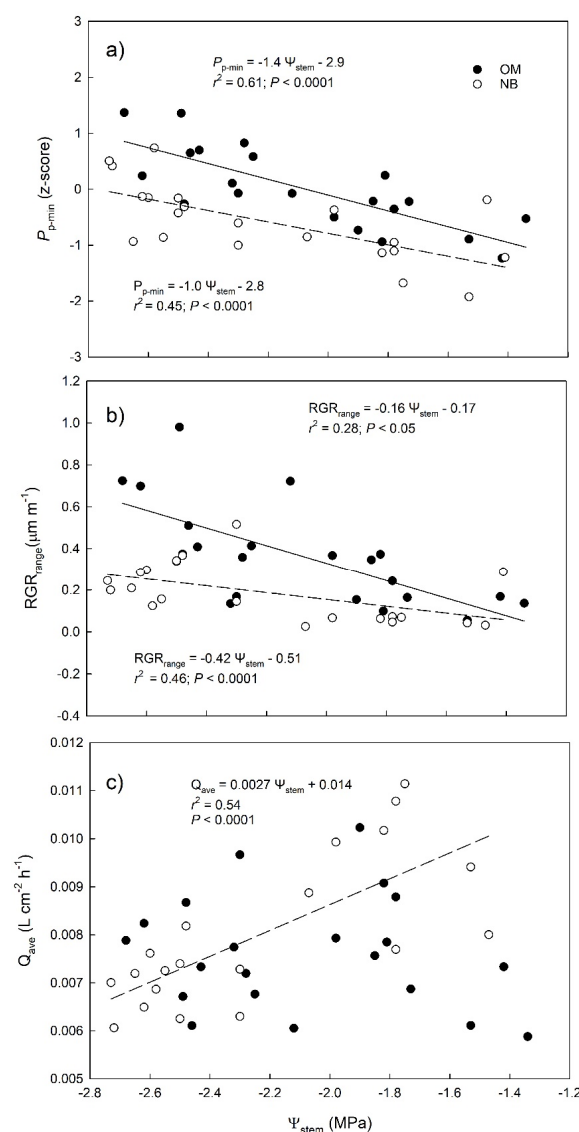

Figure 7. Relationships between midday stem water potential (Ψ_{stem}) and the daily minimum leaf patch clamp pressure (LPCP) probe outputs value ($P_{p\text{-min}}$, z-scores, panel **a**), difference between daily minimum and maximum relative growth rate (RGR$_{range}$, panel **b**), and the daily average of hourly sap flow rate (Q_{ave}, panel **c**) in the olive cultivars Olivo di Mandanici (OM, solid lines) and Nocellara del Belice (NB, dashed line).

Table 1. Comparisons of the regression slopes for the different relationships reported in Figure 6 for: daily minimum leaf patch clamp pressure (LPCP) probe outputs value ($P_{p\text{-min}}$), difference between daily minimum and maximum relative growth rate (RGR_{range}) or the daily average of hourly sap flow rate (Q_{ave}) versus midday stem water potential (Ψ_{stem}) between the two olive cultivars (Olivo di Mandanici and Nocellara del Belice).

Parameter	Slopes	SE	d.f.	t Ratio	*p* Value
$P_{p\text{-min}}$	−0.389	0.371	38	−1.049	0.301
RGR_{range}	−0.259	0.118	38	−2.195	0.034
Q_{ave}	−0.003	0.0009	37	−3.444	0.001

3.4. Hourly Dynamics of Leaf Turgor, Sap Flow, and Fruit Relative Grow Rate

To better capture the synchronism of the diurnal kinetics of water flow in stem, leaves, and fruits under mild stress as well as the differences between the two cultivars, we show a six-day snapshot, corresponding to a period between two consecutive irrigation events, from 15 to 21 August (Figure 8). Similar trends were observed in other periods between irrigation events along the course of the experiment. P_p was reported as $1 - P_p$ to make its changes directly related to changes in turgor pressure (P_c) and fruit RGR. This allows an easier comparison of the dynamics of the different indicators.
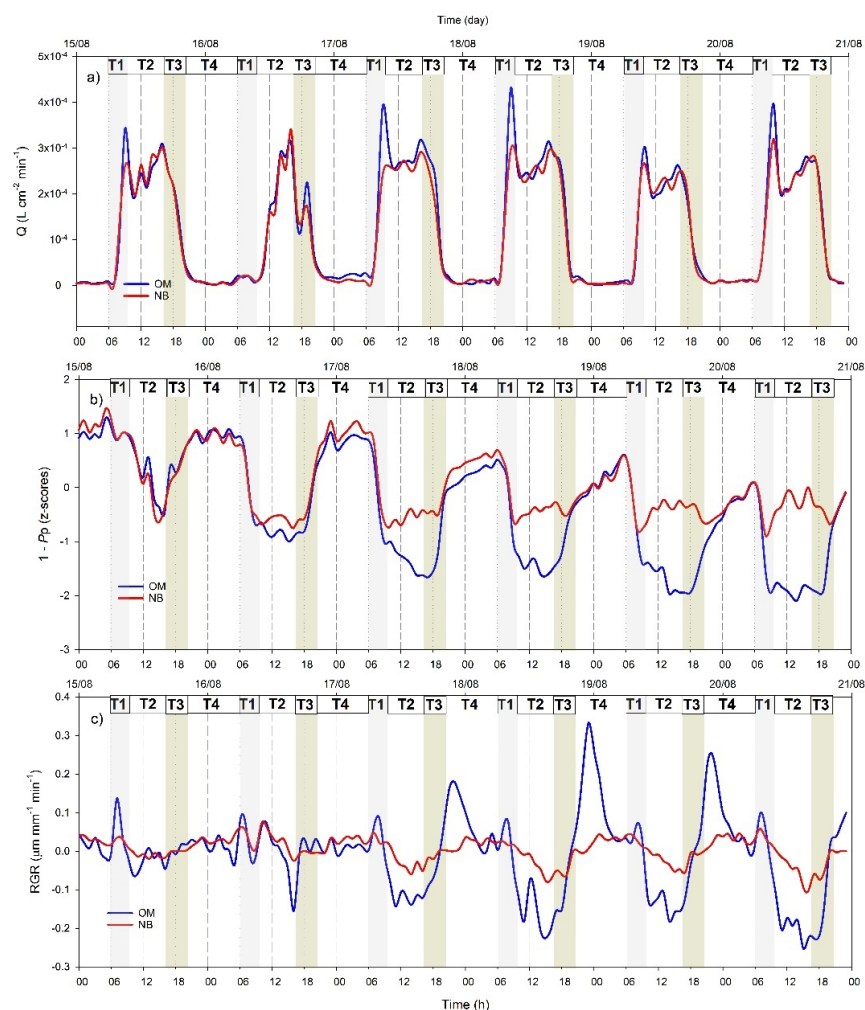

Figure 8. Hourly trends of (**a**) sap flow rate (*Q*), (**b**) standardized leaf patch clamp probe outputs ($1 - P_p$) and (**c**) fruit relative growth rate (RGR) for the olive cultivars Nocellara del Belice (NB) and Olivo di Mandanici (OM) between two consecutive irrigation events, from 15 to 21 August. T1, T2 T3, and T4 represent key timeframes described in the text.

The daily trends of P_p, Q, and RGR were constant for the first two days of irrigation withholding and after that they started showing clear changes in their daily dynamics.

Based on the hourly dynamics of the three indicators under stress (from 17 to 21 August), four key timeframes were identified.

T1 (from ~6:00 to 10:00 h): stomata open and tree transpiration starts to increase until reaching a peak, which was more pronounced in OM than in NB. Leaf turgor decreased in both cultivars in response to stomatal opening while fruit RGR kinetics were more variable. OM showed an initial increasing trend of RGR in response to the beginning of transpiration followed by a sharp decrease to values of ~ -0.2 μm mm^{-1} min^{-1}, corresponding to the transpiration peak. NB showed milder and constant decrease of fruit RGR through the whole period.

T2 (from ~10:00 to 17:00 h), characterized by a midday depression of transpiration, accompanied by an apparent recovering of leaf turgor in NB and a slowdown of leaf turgor decrease rate in OM, and by a new increase of fruit RGR in OM.

T3 (from ~17:00 to 20:00 h), when transpiration decreased constantly until it reached zero. In this timeframe, in OM, a sharp increase in leaf turgor and fruit RGR was observed, while in NB, fruit RGR increased slightly at a slower rate reaching values around zero while leaf turgor decreased slightly.

T4 (from ~20:00 h to 8:00 h), during nighttime when stomata were closed, transpiration was absent. Fruit RGR of OM decreased reaching values close to zero and leaf turgor continued to increase but at a slower rate than in T3. In NB, fruit RGR was constant and leaf turgor values continued increasing similarly to OM.

4. Discussion

4.1. Use of Multiple Continuous Sensors Improves Mild Stress Detection

The analysis of the collected data confirmed our first hypothesis that a combination of physiological parameters provides better indications of mild water stress than individual indices. In fact, even if moderate-to-high water stress was not detected during most of the experimental period by the P_p trends, a commonly used indicator derived from the PLPC (i.e., no inversion was detected in the P_p daily curves), the relationships between continuously measured parameters clearly changed as soil water availability decreased between two irrigation events, highlighting physiological responses of both olive cultivars to reduced water availability.

For example, in the period between two irrigation events, P_p daily minimum values increased continuously, indicating a gradual decrease of the daily maximum leaf turgor pressure at night (Figure 4), since trees were not able to fully recover the water lost during the day [25]. The increase in P_p daily minimum values has been previously described as an indicator of mild water stress in olive [13], as also supported by the significant relationship we observed between this indicator and stem water potential (Figure 7a). Similarly, the RGR daily range increased strongly with irrigation withholding, and it was also highly correlated with tree water status (Figure 7b), highlighting a pronounced fruit shrinkage in response to water stress as previously reported in several crops [26–30] and, most recently, also in olive [7,31]. Changes in sap flow density fluxes between irrigation events were less noticeable than those in leaf P_p and fruit RGR, most probably because of the high impact that the environment (namely VPD) has on this parameter [32], further affected by tree water status [8,33]. However, both cultivars responded to drought stress by modifying the daily dynamic of transpiration rates, which showed a high peak in the morning after a few days of water withholding (Figure 6), as also previously revealed by stomatal conductance values [7]. This is a drought resistance mechanism since plants try to take advantage of favorable environmental condition in the morning (lower VPD and better plant water status). On the contrary, in the middle hours of the day, trees closed their stomata to prevent harmful water loss and this was reflected in a midday depression of transpiration fluxes, also observed in both genotypes in response to stress.

Overall, this highlights once again the limitation of stress-based measurements that do not integrate the multiple physiological responses of trees to drought stress. For example, a single measurement of stomatal conductance at 10:00 h would indicate no stress, due to the transpiration peak, while the multiple continuous measurements revealed that the high transpiration rates at that time of the day are an adaptation to reduced water availability. Hence, the use of only one indicator would have led to misleading conclusions, of difficult interpretation, as pointed out by recent studies [34].

4.2. Different Physiological Responses of the Two Genotypes to Water Withholding

The use of several continuous sensors was also critical to detect and understand the different physiological responses of the two genotypes to water withholding. Under stress, a more pronounced peak of transpiration was exhibited by OM. The higher morning water flux (and gas exchange) compensated for the stomatal closure later in the day, resulting in a lack of correlation between the average sap flow values and Ψ_{stem} in this cultivar (Figure 7c). Conversely, the relationship between the daily average value of sap flow density and stem water potential in NB was highly significant and positive (Figure 7c), indicating that this cultivar responded to a reduction in available water (more negative Ψ_{stem}) by closing stomata and reducing water fluxes (lower Q_{ave}). This is generally one of the first and most sensitive responses of plants to drought [35] that has been shown to vary in different olive cultivars [36].

The high rates of transpiration attained by OM are also reflected in the relatively high degree of shrinkage of both leaf and fruit tissues observed for this cultivar under drought stress. In this cultivar, a sharp drop of leaf turgor and fruit RGR was observed during the morning transpiration peak. In NB, the morning peak of sap flow was less pronounced (Figure 8a), the minimum leaf turgor measured during the day (maximum P_p) was constant (Figure 4a), and the fruit RGR drop was less pronounced (Figure 5b), suggesting a very different response of this cultivar to dehydration. The analysis of the detailed daily water flux kinetics in Figure 8 highlights that the lack of turgor decrease in NB leaves during the day is due to a partial re-increase of P_p values at midday, a phenomenon called semi-inversion and associated with the decrease of turgor pressure (P_c) to values close to the turgor loss point [12].

Hence, as hypothesized, the two cultivars showed different responses to water stress, including (1) a higher tendency of OM leaf tissues to shrink relative to NB, (2) a lower tendency of OM to invert the P_p curves when leaves are close to the turgor loss point than NB (Figure 3) and (3) a smaller drop in Ψ_{stem} for OM than NB (Figure 3). Such a difference in Ψ_{stem} behavior between the two cultivars has been reported in previous studies [7,13]. All these three responses of OM are typical of genotypes with more elastic tissues [37], or higher capability to adjust tissue elasticity [38], pointing to this mechanism as a potential adaptive strategy of this cultivar to cope with dehydration.

4.3. Cultivar Dependency of Continuous Indicator Sensitivity

The P_p readings depend on the capability of the leaf to transfer the pressure applied by the sensors' metallic clamps, which is governed by changes in cell volume [12]. The correlation between P_p and P_c is hence based on the assumption that volume changes are directly related to P_c, and tissue elastic modulus is linearly dependent on P_c. This assumption implies that differences in the elastic properties of the tissues may play an important role in dictating the sensitivity of the LPCP probe to turgor, leading to the very different dynamic of maximum P_p values between NB (constant) and OM (increasing) in response to drought stress. This suggests that tissue mechanic properties should be taken into consideration in the development of irrigation strategies based on LPCP. For example, the inversion phase of the P_p curve, which allows detecting mild stress in 'Arbequina' [10,11], is not a good indicator of mild stress for cultivars that exhibit physiological responses to drought similar to OM.

The different drought resistance mechanisms exhibited by the two cultivars are also well depicted by the analysis of fruit RGR. While in other species, such as peach, transpiration from the cuticle has a great influence on fruit shrinkage [39], in olive, the fruits have few stomata that are rapidly covered by wax at early stages of development, hence we can assume that loss of water from the cuticle is negligible. In such conditions, we suppose that the high rate of fruit shrinkage observed in the daytime in OM is highly related to water backflow to leaves [16,40–42].

Interestingly, shrinking and swelling in NB fruits were very limited, and fruit RGR_{range} was a weaker predictor of mild water stress than in OM (Figure 7b). A lack of correlation between fruit RGR daily dynamics and plant water status is generally associated with a partial decrease of the hydraulic conductivity of the vascular pathway connecting the fruit and the rest of the plant, causing hydraulic isolation of the fruit [43]. However, an active accumulation of solutes, as previously observed in olive fruits [44], may also play an important role in decreasing fruit osmotic potential in NB and maintaining a more stable volume throughout the day. On the contrary, in OM the daily water flux kinetics highlights quick changes of fruit RGR in response to changes in stomatal aperture, e.g., in the morning before the transpiration peak and at midday during the decrease of transpiration fluxes, suggesting a high dependence of the fruits from the parent plant. Hence, fruit sensing seems to be a better choice in OM, since it is more representative of tree water status.

Overall, this study highlights the limitation of stress-based studies that do not consider cultivar specific drought resistance mechanisms.

5. Conclusions

The results of this study confirm that monitoring continuously leaf, fruit, and stem water dynamics can help detect desirable water stress levels in olive earlier than a single commonly used indicator of water stress such as the daily P_p curves. Before the P_p daily curve shape (suggested indicator of stress for 'Arbequina') detected any stress, trees had already developed physiological changes to deal with the reduced water availability affecting water relations between organs, and probably assimilation and growth.

In OM, fruits acted as sinks of water during the night and as water sources during the day, leading to a strong daily shrinkage and swelling pattern in response to plant stress. This highlights the important and still unrevealed role that fruits have as water storage compartments in drought resistance mechanisms of olive.

Overall, the results encourage the adoption of cultivar-specific combinations of plant-based continuously monitoring sensors for precise management of irrigation in intensive olive systems. More studies should be performed on this topic, also considering the difficulty to replicate continuous measurements on large numbers of trees. Other limitations of the sensors' applicability to commercial orchards and their specific pro and cons have been well discussed by Fernández [45]. In particular, this study highlights that the type and magnitude of plant physiological responses to drought influence the capability of plant-based indicators to detect stress. However, such responses can be affected within the same genotype by other factors such as crop load, previously experienced stress, or phenological stage, so that further work should be done to integrate all this information in simple grower-friendly protocols, that can be developed for an efficient agricultural water management. This becomes of paramount importance in environments with reduced water availability and the need to maintain, for productive purposes, relatively high levels of plant hydration during drought-sensitive phenological stages.

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