

Article

Ecophysiological Recovery of Micropropagated Olive Cultivars: Field Research in an Irrigated Super-High-Density Orchard

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Abstract: This research focuses on the seasonal patterns of the ecophysiological recovery of four olive cultivars (Arbequina, Coratina, Frantoio, and Urano), both micropropagated and self-rooted, grown in a mature, irrigated, super high-density (SHD) orchard under Mediterranean conditions (Southern of Italy). The aim was to observe the impact of the micropropagation method on the ecophysiological responses. Ecophysiological parameters, including leaf water potential (LWP), stomatal conductance (gs), net photosynthetic rate (Pn), and transpiration rate (E) were assessed. Self-rooted trees consistently exhibited superior gs, E, and Pn recovery compared to the micropropagated ones. ‘Arbequina’ maintained elevated levels of Pn under water-deficit conditions. ‘Coratina’ exhibited increases in gs and E after irrigation. ‘Frantoio’ demonstrated recovery capabilities, with lower LWP and higher Pn under stress. ‘Urano’ micropropagated trees achieved higher gs values in mid-summer, while self-rooted trees sustained higher Pn later in the season. This field research highlighted the important role of the propagation method in optimizing the physiological performance of olive cultivars in SHD orchards. Furthermore, it highlighted the necessity of long-term studies on the effects of propagation methods and their interactions with other farming practices.

Keywords: drought stress; irrigation management; micropropagation; mist propagation; olive ecophysiology; crop management; super high-density orchard



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1. Introduction

The Mediterranean basin has always had a long history of olive tree cultivation, influencing the economy, trades, and local traditions; nevertheless, much of this region’s areas are semi-arid environments characterized by high evapotranspiration demand and low precipitation [1]. Water availability strictly influences plant physiology; plants respond to water availability through several mechanisms to maintain cell turgor and optimize water-use efficiency [2,3]. The Olive tree (*Olea europaea*, L.) exhibits significant adaptability to water availability changes through different physiological mechanisms to address drought stress, such as stomatal closure, osmotic regulation, and resistance to cavitation, which help preserve water under high evaporative demand [4]. Olive trees can maintain photosynthetic activity and transpiration even under water deficit conditions by lowering tissue water content and setting a high potential gradient between leaves and roots [5]. However, olive production faces challenges, particularly environmental stresses, including those related to water availability. Every year, water scarcity is a challenge to face using innovative methods [5–8].

The olive sector is confronting several challenges associated with climate change and is in need of urgent mitigation measures. Rising temperatures and declining precipitation significantly affect olive tree performance, and impact photosynthesis, respiration, phenology, water use, and yield [9]. For this, the development of robust adaptation and mitigation

strategies are required. Italy, a major olive producer, is facing a crisis characterized by shrinking cultivated areas, reduced production, and limited innovation. This situation is exacerbated by traditional practices and phytosanitary issues like *Xylella fastidiosa*, further aggravated by climate change and drought [10].

For instance, optimal olive flowering is closely tied to specific temperature ranges, with future warming likely to disrupt these conditions, leading to reduced productivity and quality [8]. In Tunisia, the phenological behavior of native olive cultivars is already showing variability due to local climatic conditions; warmer winters are advancing the growing season and increasing the risk of pollination disruption and insect attacks [11]. Given these interconnected challenges, it is crucial to design and implement adaptation measures using sustainability principles, considering inherent uncertainties and global-scale changes in technology and economics [12].

In this context, ecophysiological parameters such as leaf water potential, stomatal conductance, net photosynthetic rate, and transpiration are highly relevant to determining the response of olive trees to water stress conditions. Leaf water potential, as stem water potential (Ψ), is a key indicator of the plant's water status and varies according to the environmental and physiological conditions of the olive tree [1]. For example, cultivar (cv) Coratina shows significant variability in response to water stress, with Ψ values dropping to -6.45 MPa during extreme drought periods [13]. Leaf water potential significantly decreased under deficit irrigation conditions, highlighting the importance of careful water management to maintain adequate leaf water potential levels and prevent physiological damage [14]. 'Frantoio', on the other hand, tends to maintain relatively high water potential, rarely dropping below -3.5 MPa, demonstrating greater resilience and recovery ability after stress periods [15]. 'Arbequina' is particularly tolerant to water stress, capable of maintaining photosynthetic activity even at low Ψ values, which can reach -6.5 MPa under severe stress [16]. 'Arbequina' ability to maintain a more stable leaf water potential is attributed to osmotic adjustment mechanisms that allow the plant to retain cell turgor even under extreme stress conditions [7,16,17].

Stomatal conductance is a fundamental parameter reflecting the plant ability to regulate gas exchange and transpiration. In cv Coratina, stomatal conductance decreases significantly with increasing water stress, reaching very low values around $0.03 \text{ mol m}^{-2} \text{ s}^{-1}$ under severe stress [13]. During periods of water stress, stomatal closure represents a crucial defense mechanism to prevent water loss, but this process also limits CO_2 uptake, thereby reducing photosynthesis. 'Frantoio' demonstrates a capacity for stomatal regulation that helps mitigate the effects of stress, although it shows a reduction in stomatal conductance under water stress. Under deficit irrigation conditions, stomatal conductance can drop to $0.06 \text{ mol m}^{-2} \text{ s}^{-1}$ but it tends to recover quickly as water conditions improve [18]. 'Arbequina' exhibits greater flexibility in stomatal conductance, maintaining sufficient values to allow acceptable photosynthetic activity, even under moderate stress, but drastically reduces stomatal conductance under severe stress [19–22]. The ability of cv Arbequina to regulate stomatal conductance is particularly important in super-intensive cultivation systems, where optimal irrigation management is a prerogative in maintaining high productivity levels.

Net photosynthesis is closely linked to water status and stomatal conductance. 'Coratina' experiences a drastic reduction in water stress due to the reduction in stomatal conductance and photosynthesis, although deficit irrigation strategies can partially mitigate these effects, maintaining acceptable levels of net photosynthesis [20]. Previous studies have shown that, despite seasonal variations, cv Coratina can maintain active photosynthesis through adaptation strategies that optimize the use of available water [23]. 'Frantoio' also shows a decrease in net photosynthesis under water stress conditions but maintains some photosynthetic activity thanks to regulatory mechanisms that optimize the use of available water [24]. 'Arbequina', known for its tolerance to water stress, can maintain relatively high levels of net photosynthesis even under deficit conditions, thanks to osmotic adjustment mechanisms that allow the plant to maintain cell turgor and photosynthetic efficiency.

Transpiration is the process through which plants lose water as vapor through stomata and is essential for nutrient transport and leaf cooling. In cv Coratina, transpiration decreases significantly under water stress, following the trend of stomatal conductance, as an adaptation mechanism to limit water loss [19,20]. Moreover, despite the reduction in transpiration, cv Coratina can maintain adequate water balance through stomatal regulation and internal water redistribution [21]. Similarly, in cv Frantoio, transpiration decreases under water stress, but the cultivar shows an ability to maintain sufficient photosynthetic activity while limiting water loss. 'Arbequina' follows a similar pattern, with reduced transpiration under severe water stress while maintaining sufficient photosynthetic efficiency to ensure trees' survival. It is already known that fully irrigated trees exhibit superior growth and yield compared to those under limited water supply, but the influence of irrigation on olive tree water status and gas exchange emphasizes the critical role of adequate water supply during key developmental stages [13,22,25]. These physiological adaptations are fundamental for irrigation management and the sustainability of olive production in arid and semi-arid environments.

The economic and environmental advantages of innovative planting systems and micropropagation in olive cultivation are mandatory for enhancing long-term competitiveness and sustainability. Super high-density (SHD) planting systems demonstrate higher yield per hectare, offering economic benefits despite lower yield per tree compared to low-density systems [26]. Using the correct deficit irrigation can improve water needs, reduce vigor, maintain high yields, and refine olive oil quality [27,28]. These systems support mechanization, reduce labor costs, and increase efficiency. The transition from low-density (200 trees/ha) to SHD systems (>1200 trees/ha) has shown a lower global warming potential (GWP) per ton of olives, making them environmentally advantageous [29]. Moreover, applying the right techniques can assure higher yield levels and oil quality; row-orientation is one of these techniques and allows for incrementation of yields with the same inputs, as well as row spacings [30–33]. In areas where new and emerging pathogens are becoming more and more relevant, such as the area of south Apulia with the bacterium *Xylella fastidiosa* subsp. *pauca*, the applications of these technologies, combined with new cultivars, can give incredible results [34–36]. The SHD planting system, which requires low-vigor cultivars, was first tested on varieties such as 'Arbequina' and 'Arbosana' [32,37,38]. This system, widely spread over 400,000 ha in the world and recently applied to other fruit tree species like almond [39–41], pushed the breeders to patent new cultivars, like 'Urano', 'Oliana', and 'Lecciana' that have shown adaptability to SHD planting systems [36,42,43]. Conversely, traditional olive cultivars such as 'Coratina', 'Leccino', and 'Frantoio' exhibit significant vegetative and productive limitations under these cropping conditions [37,44–46].

The propagation method of fruit tree crops is becoming more important year by year [47–50]. Significant efforts have been directed towards enhancing olive plant production through in vitro protocols [51], including the integration of mycorrhizal fungi [52], varying nutrient levels [53,54], and different light exposures [55]. Micropropagation techniques, particularly in vitro culture, enhance the propagation rates of economically valuable cultivars like 'Arbequina', ensuring genetic stability and true-to-type plants, which are essential for maintaining high-quality production [56]. These micropropagated trees exhibit well-distributed root systems and regular canopy shapes, contributing to better field performance and productivity [45].

Understanding the impact of new in vitro propagation techniques on the morphology and architectural parameters of different olive cultivars is vital for assessing their suitability for intensive olive orchards [45]. Micropropagation offers a cost-effective and simplified method for propagating olive varieties that are difficult to root with traditional methods and, so far, no significant genotype or phenotype differences have been reported between in vitro and mist-propagated trees [57,58]. Over the past few decades, scientists have focused on understanding the effects of this method on olive trees. Literature indicates that micropropagated trees of 'Nocellara etnea' and 'Carolea' do not exhibit juvenile features, and begin producing fruit in the second and third growing seasons, respectively. Addi-

tionally, acclimatized 'Frantoio', 'Canino', 'Leccino', and 'Moraiolo' trees can differentiate flower buds even during in-pot development [59]. Studies have shown that in vitro olive trees do not have a strict juvenile phase, although fruit production is slightly delayed. Micropropagated olive trees delay the onset of flowering by only one year, and they exhibit rapid post-implant growth with production times comparable to those propagated with cuttings [45,60].

The primary factors affecting fruit tree productivity are flowering and fruit set, which are especially critical for olive trees due to their biennial bearing and delicate balance between vegetative and reproductive growth stages [61]. The propagation method significantly influences fruiting dynamics. Branching dynamics showed notable differences influenced by the propagation method [62]. Micropropagation enhances vegetative growth in 'Coratina' but not in 'Arbequina', confirming the suitability of Micro trees of cv. Arbequina for SHD systems, as indicated by previous studies [62]. Evaluating the vegetative and fruiting characteristics of new and traditional cultivars allows the development and promotion of new growing models to meet the demand for innovation and competitiveness in the sector [36,44]. Moreover, the method of propagation and the specific treatments applied can drive the success of the orchard, determining the physiological outcomes in olive cultivation. Unfortunately, until now, no research has been done on the field ecophysiological behavior of micropropagated olive trees.

The aim of this field research work is to investigate the seasonal ecophysiological recovery patterns of four olive cultivars grown in an adult, irrigated, super high-density orchard, focusing on the impact of micropropagation methods on their physiological responses. By examining parameters such as stomatal conductance, leaf water potential, transpiration, and net photosynthetic rate, the study aims to optimize both orchard planting and irrigation management to enhance olive tree productivity and resilience under varying water conditions.

2. Material and Methods

2.1. Site and Climatic Conditions

The trials were carried out in an irrigated super high density olive orchard at the experimental farm "Martucci" of the University of Bari Aldo Moro, located in Valenzano (41°01'45.6" N; 16°53'50.3" E, 105 m a.s.l.; Apulia region, Southern Italy). The olive trees were spaced 4.0 m × 1.5 m apart (1660 trees per hectare) with north–south (N–S) row orientation; the trees were trained as central-leader form. The soil was sandy–clay (sand, 630 g kg^{−1}; silt, 160 g kg^{−1}; clay, 210 g kg^{−1}), classified as a Typic Haploxeralf (USDA) or Chromi-Cutanic Luvisol (FAO). The climate was Mediterranean, with mild winters and hot summers; Köppen and Geiger classify as "Csa" (<https://en.climate-data.org/europe/italy/apulia/valenzano-14029/>, accessed on 5 June 2024). The average annual temperature is about 16 °C and the annual rainfall is about 620 mm; most of the rain falls in autumn–winter, and the warmest and driest month is August (<https://en.climate-data.org/europe/italy/apulia/valenzano-14029/>, accessed on 5 June 2024).

2.2. Plant Material and Experimental Design

The experiment was conducted on four olive cultivars: Arbequina, Coratina, Frantoio, and Urano; the olive trees, 4 years old, belonged to two different propagation methods: self-rooted (SR) and micropropagated (MP). SR plants were obtained from semi-hardwood cuttings [63], while MP were obtained with M268 as proliferation medium (OM + zeatin 1.0 mg/L, mannitol 36 g/L; agar 0.7%; pH 5.7) and R141 as rooting media (1/4 MS IBA 0.8 mg/L; putrescine 160 mg/L; sucrose 20g/L; agar 0.7%) [64]. The experimental design used was complete randomized blocks.

2.3. Agro-Climatic Parameters

The agrometeorological variables were recorded through the "Bollettini meteorologici regionali mensili" of the Apulia region. In particular, minimum, maximum, and mean

temperatures per each per day were recorded. Moreover, the distribution and frequency of rainfall, which are critical for understanding water demands and irrigation needs for crops, were also evaluated.

2.4. Irrigation and Crop Management

All the trees were subjected to the same water deficit regime by irrigating whenever the readily available soil moisture, set at 67% of the total available moisture, was depleted in the deep fraction of the soil between 0.20 m and 0.40 m. Irrigation was scheduled by monitoring the soil water potential (Ψ_s) every 3 days at the soil depth considered to restore the readily available moisture; Ψ_s was monitored using a potentiometer (WP-4T, Decagon Devices, Inc., Pullman, WA, USA). Props, drip irrigation, and routine cultural practices (nutrition, weeds, and disease control) were set up as already described [65] and following the guidelines of Integrated Pest Management (IPM).

2.5. Ecophysiological Parameters

During the irrigation season, from June to September, data related to the eco-physiological parameters were acquired for each cultivar (cv) and for each propagation method (PM) one day before the irrigation and one day after in order to observe trees recovery behavior (R). Measurements were taken at midday (11:30 to 13:00 solar time) at light saturation ($\text{PAR} \geq 1600 \text{ mol photons m}^{-2} \text{ s}^{-1}$) on 2 leaves of 3 representative trees per treatment. Leaf samples were healthy, mature, fully expanded, and well lit. The eco-physiological parameters acquired were stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), net photosynthetic rate (P_n ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and leaf water potential (LWP; MPa); g_s , E , and P_n were measured using a gas exchange system (LCA-4, Analytical Development Company, Ltd., London, UK); LWP was measured through the same potentiometer used for monitoring Ψ_s .

2.6. Statistical Analysis

Analysis of Variance (ANOVA) was performed to assess differences among treatments and interactions; the factors analyzed for the interaction effect were R, cv, and PM. Tukey's post-hoc test was applied following significant ANOVA results to separate group differences. The Pearson correlation test was used to test the correlation between the parameters. Data were analyzed and represented by using Spyder (© 2023 Spyder, Version 5.4.3 for Windows) and SigmaPlot (Systat Software Inc., Chicago, IL, USA, Version 14 for Windows).

3. Results

3.1. Agrometeorological Data and Irrigation

Throughout the year, recorded temperatures exhibited a clear seasonal pattern, in line with other years (Figure 1). The annual average temperature reflects this seasonal trend, with peak values around 30 °C in the summer months and lows of about 5 °C in the winter months.

During the winter months, particularly in January and February, the lowest minimum temperatures were observed, dropping to −5 °C. In August, the hottest month of the year, maximum temperatures often exceeded 35 °C, reaching a peak of over 40 °C. These seasonal fluctuations are indicative of the typical conditions of a temperate climate, providing important insights for sectors such as agriculture, energy resource management, and climate change studies.

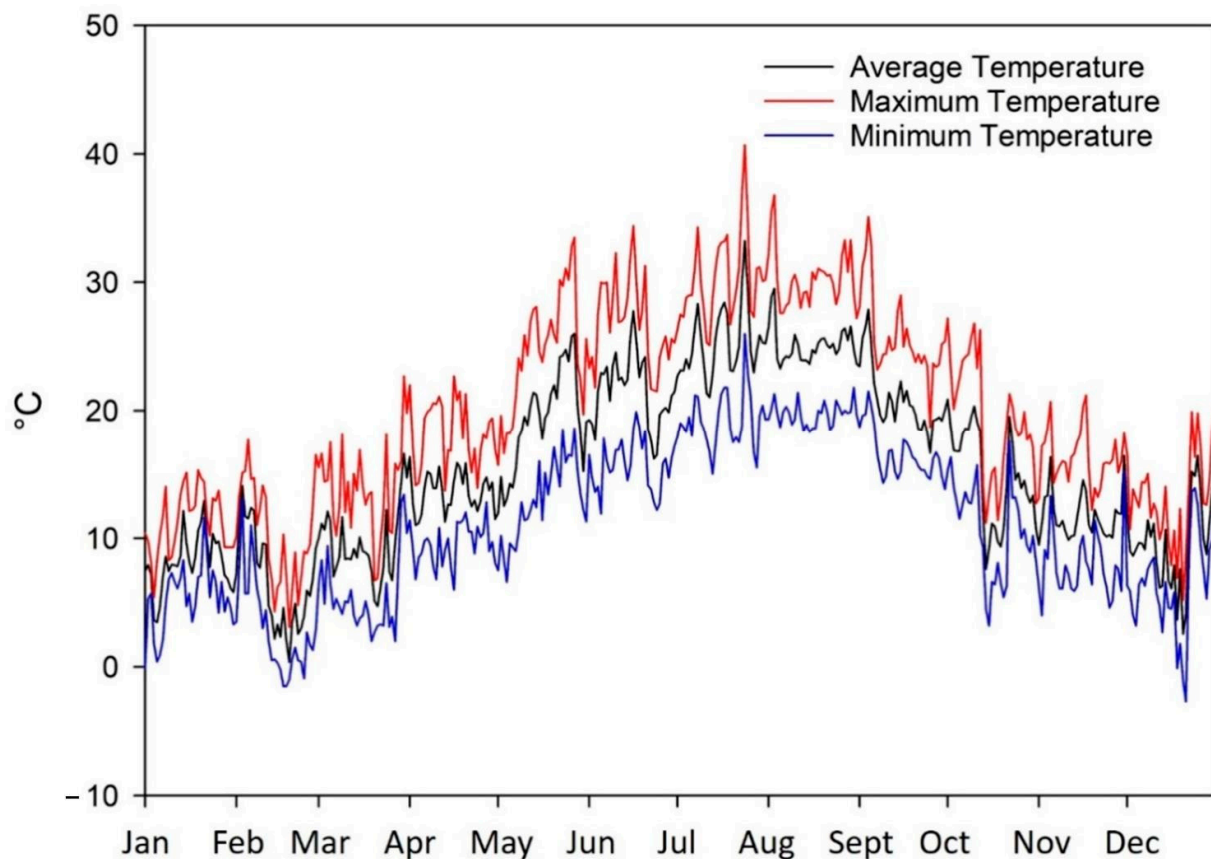


Figure 1. Daily variation of maximum, minimum, and average temperatures in the experimental area during the year of the trial.

Figure 2 reveals that ET_c values were starting to rise significantly from April and peaked during June and July. This period of increased ET_c reflects the heightened water demand by crops due to higher temperatures and longer daylight hours. Conversely, rainfall was more frequent and abundant in the early months (January to March), with a marked reduction during the peak summer period, which coincided with the highest ET_c values. Rainfall increased again in the autumn months, especially in October, before declining towards the end of the year. This seasonal pattern highlights potential water stress for crops from June to August, indicating the necessity for irrigation during these months to meet crops' water needs. The periods of higher rainfall and lower ET_c, such as early spring and autumn, suggest a positive water balance, reducing the need for supplementary irrigation. Understanding these patterns provides the possibility of realizing irrigation planning and water resource management in agriculture, ensuring optimal crop growth and yield in regions with similar climatic conditions.

The rainiest month was October, while the driest was June. The hottest month was July, and the coldest month was December; over the irrigation season, when measurements were acquired, the driest months were July and August (Figure 1). The irrigation season started in May, at the flowering phase (code 65, BBCH scale [66]) and ended in September, corresponding to the beginning of the veraison phase (code 81, BBCH scale; [66]). The seasonal irrigation volume was 1840 m³ ha⁻¹, with an irrigation volume applied at each irrigation of 60 m³ ha⁻¹, and a mean irrigation interval of 3 days.

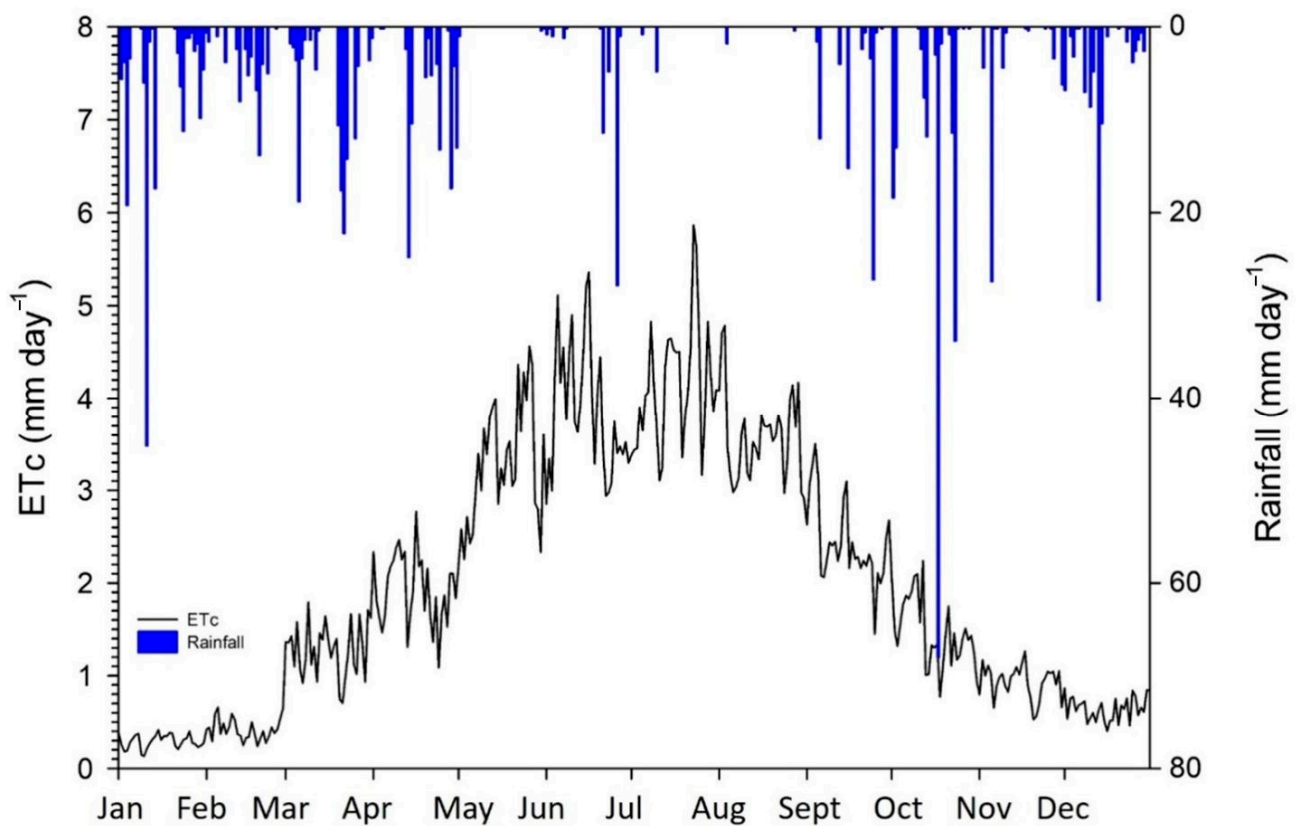


Figure 2. Daily trend of the crop evapotranspiration calculated following the Hargreaves-Samani equation (1985), FAO-56 crop coefficients, and rainfall during the year of the trial.

3.2. Interactions

The parameters showed a variable influence of recovery (R), cultivar (cv), and propagation method (PM) (Table 1). Recovery showed the highest significance, impacting all the examined ecophysiological parameters; cv was highly relevant for LWP and E, and PM slightly influenced LWP and gs.

Table 1. Effect of recovery (R), cultivar (cv), propagation method (PM), and their interactions on the ecophysiological variables measured on the olive trees (LWP, leaf water potential; gs, stomatal conductance; E, transpiration rate; Pn, net photosynthetic rate). n.s. = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

	LWP	gs	E	Pn
R	***	***	***	***
cv	***	n.s.	**	n.s.
PM	*	*	n.s.	n.s.
R × cv	n.s.	n.s.	n.s.	n.s.
R × PM	n.s.	n.s.	n.s.	n.s.
cv × PM	*	n.s.	n.s.	n.s.
R × cv × PM	n.s.	n.s.	n.s.	n.s.

Notably, for LWP, the interaction between the cultivar and propagation method was significant ($p = 0.041$), which suggested that the effect of the cultivar on LWP was dependent on the propagation method. The other two-way interactions and all the three-way interactions did not reach statistical significance.

3.3. Ecophysiological Recovery

Considering that the most relevant differences were specifically for R, attention was focused on this particular parameter, and we discuss the results for each cultivar.

3.3.1. ‘Arbequina’

LWP values were generally less negative post-irrigation compared to pre-irrigation, indicating improved water status in the trees following irrigation, with significant differences (Figure 3A,B). In MP, statistical differences were observed in every DOY except for the second date. The highest difference was observed in DOY 245–247, with almost 1 MPa between pre- and post-irrigation (−4.01 and −3.23 MPa, respectively). For ‘Arbequina’, SR showed a different pattern. In fact, post-irrigation data showed higher values only in DOYs 245–247 (−4.77 and −3.48 MPa for pre- and post-irrigation, respectively). For this parameter, no statistical differences were observed between the two propagation methods either pre- and post-irrigation.

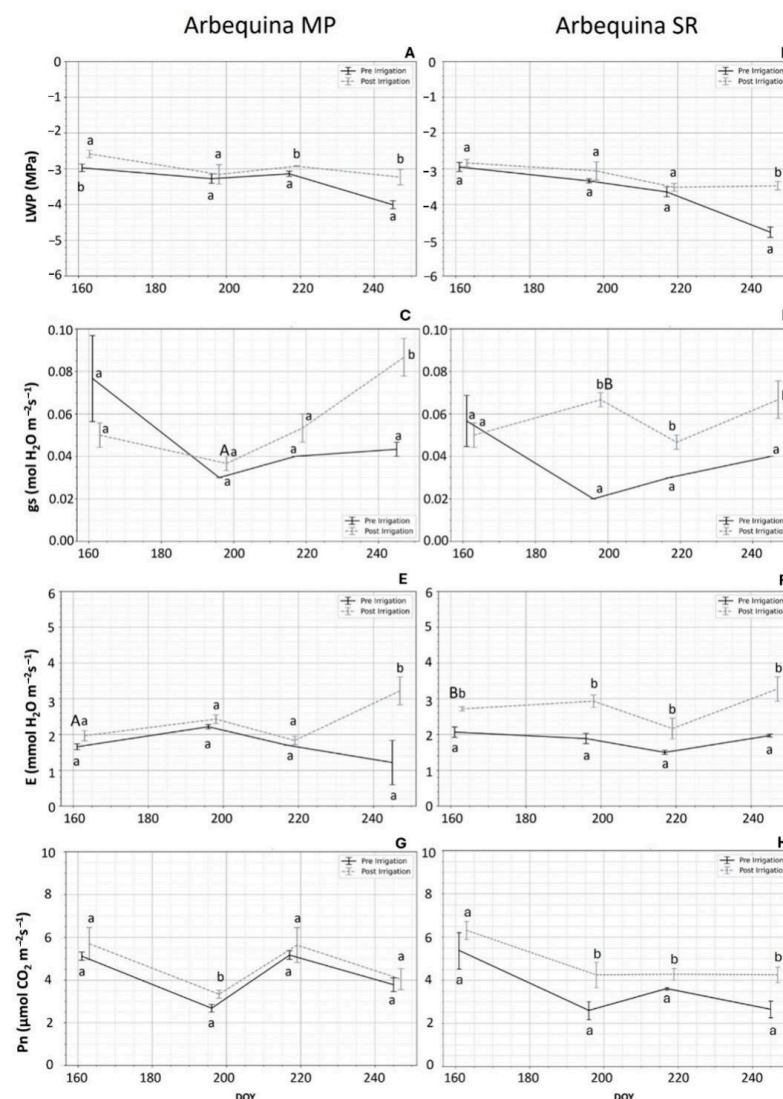


Figure 3. Mean and standard error of leaf water potential (LWP; MPa; (A), micropropagated; (B), self-rooted), stomatal conductance (gs; mmol H₂O m⁻² s⁻¹, (C), micropropagated; (D), self-rooted), transpiration rate (E; mmol HO m⁻² s⁻¹; (E), micropropagated; (F), self-rooted), and net photosynthetic rate (Pn; μmol CO₂ m⁻² s⁻¹; (G), micropropagated; (H), self-rooted) of micropropagated (MP) and self-rooted (SR) ‘Arbequina’. Lowercase letters indicate significant differences before and after irrigation; capital letters indicate differences among cultivars ($p < 0.05$).

Stomatal conductance (g_s) exhibited a marked difference between pre- and post-irrigation conditions for both propagation treatments and across all DOYs. MP post-irrigation g_s was consistently higher than pre-irrigation values, mainly in the last sample days (Figure 3C). In particular, relevant differences were observed at DOY 161 (0.076 and 0.033 mol H₂O m⁻² s⁻¹ for post- and pre-irrigation, respectively) with significant differences at DOY 245–247, in which g_s increased to 0.093 mol H₂O m⁻² s⁻¹ post-irrigation, significantly higher than the pre-irrigation value of 0.053 mol H₂O m⁻² s⁻¹. Different trends were observed in SR (Figure 3D): at DOY 196–198, post-irrigation showed significantly higher g_s than pre-irrigation (0.064 compared to 0.035 mol H₂O m⁻² s⁻¹). Moreover, a statistical difference was also observed for this sample day between the propagation methods, in which SR post-irrigation showed the highest value. The trend persisted throughout the other days, with significantly higher values post-irrigation.

The E followed a similar pattern to g_s , mostly for the notable increments observed post-irrigation (Figure 3E,F). In MP, post-irrigation values were consistently higher only in DOY 245–247 (3.58 and 1.54 mmol H₂O m⁻² s⁻¹ for post- and pre-irrigation, respectively), while no statistical difference was observed in the other DOYs. Different situations were observed for SR. In fact, post-irrigation showed constantly higher values than pre-irrigation throughout the season. Moreover, at DOY 161–163, SR post-irrigation showed higher values also than MP post-irrigation (2.70 and 2.00 mmol H₂O m⁻² s⁻¹, respectively).

Net Pn showed a different pattern from MP to SR (Figure 3G,H); in the first one, statistical difference was observed only in DOYs 196–198, in which post-irrigation performed better than pre-irrigation (3.34 and 2.68 μmol CO₂ m⁻² s⁻¹, respectively); in the second one, statistical differences were observed for every sample day except for the DOYs 161–163. In particular, the highest recovery was observed in DOYs 245–247, with statistically higher Pn in post-irrigation than pre-irrigation (4.24 and 2.64 μmol CO₂ m⁻² s⁻¹, respectively). Also, for this parameter, no statistical differences were observed between the two propagation methods.

3.3.2. ‘Coratina’

LWP values were less negative post-irrigation, indicating better water status for both propagation methods. In MP, even if post-irrigation values were constantly higher all season, a statistically higher value was observed only for DOY 196–198 (−3.88 and −3.28 MPa before and after irrigation) (Figure 4A). By DOY 245–247, no statistical difference was observed. SR showed a similar path (Figure 4B). In fact, statistical difference was observed in the middle of the season, with the most relevant variation being in DOY 217–219 (−4.95 and −4.09 MPa for pre- and post-irrigation, respectively). Furthermore, in DOY 198, MP showed a statistically lower LWP (−3.28 MPa) compared to SR (−3.75). The g_s of both MP and SR trees showed notable differences before and after irrigation (Figure 4C). In the MP treatment, g_s values increased post-irrigation in the middle of the season (Figure 4C). In particular, at DOY 196–198, the irrigation caused 100% better g_s compared to pre-irrigation (0.042 vs. 0.021 mol H₂O m⁻² s⁻¹); this trend was maintained throughout the rest of the season, with no statistical differences in the last measurements (0.050 and 0.031 mol H₂O m⁻² s⁻¹). For the SR trees, significant differences were also observed, especially from the second measurement until the end of the season (Figure 4D). At the end of the season, at DOY 245–247, g_s was 0.031 mmol H₂O m⁻² s⁻¹ post-irrigation, significantly higher than the pre-irrigation value of 0.019 mmol H₂O m⁻² s⁻¹; no statistical differences were observed between the two propagation methods. E displayed significant increments post-irrigation in both ‘Coratina’ MP and SR treatments. In MP, E was constantly and significantly higher post-irrigation, from the first measurement (2.08 and 2.47 mmol H₂O m⁻² s⁻¹ for pre- and post-irrigation) to the last (2.17 and 3.65 mmol H₂O m⁻² s⁻¹ before and after irrigation, respectively) (Figure 4E). In the SR treatment, significant differences were observed throughout the season, with the most relevant difference observed at DOY 196–198 (Figure 4F). In fact, post-irrigation transpiration (3.14 mmol H₂O m⁻² s⁻¹) was statistically higher compared to pre-irrigation (1.47 mmol H₂O mm⁻² s⁻¹). Moreover, statistical difference was also observed between the two propagation methods

in DOY 163, in which SR showed statistically higher values than MP (3.23 and 2.47 mmol H₂O m⁻² s⁻¹ for SR and MP, respectively).

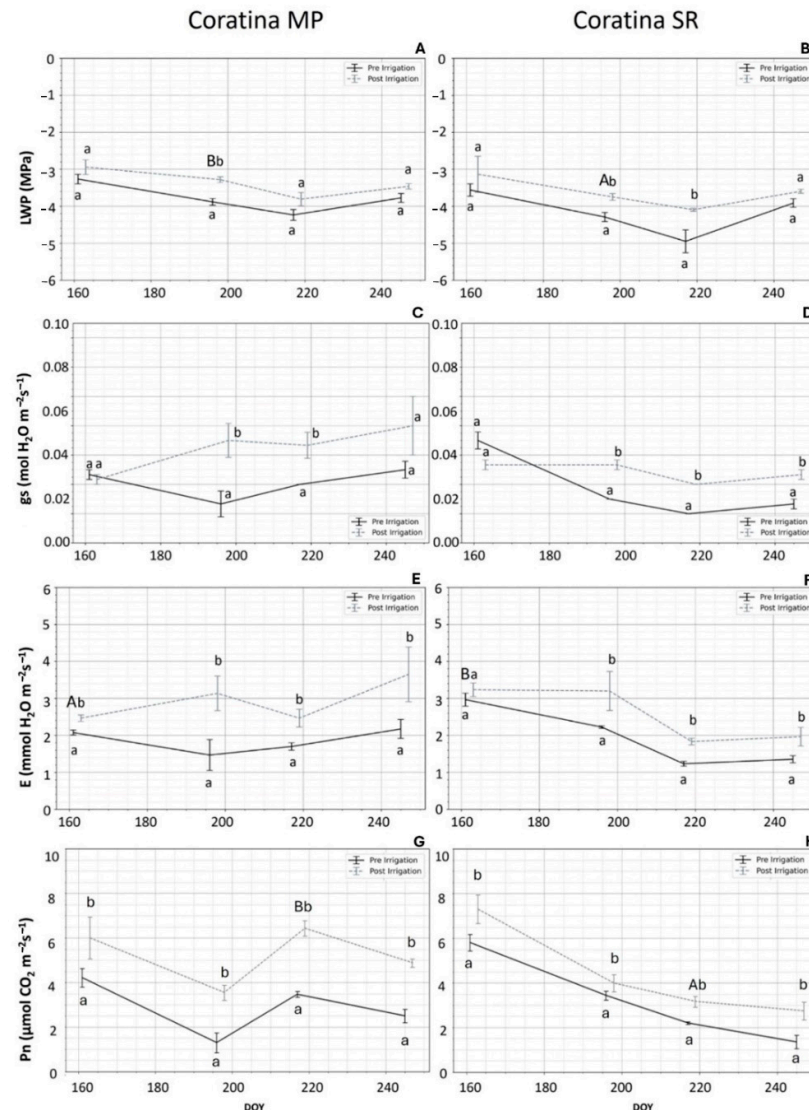


Figure 4. Mean and standard error of leaf water potential (LWP; MPa; (A), micropropagated; (B), self-rooted), stomatal conductance (gs; mmol H₂O m⁻² s⁻¹, (C), micropropagated; (D), self-rooted), transpiration rate (E; mmol HO m⁻² s⁻¹; (E), micropropagated; (F), self-rooted), and net photosynthetic rate (Pn; μmol CO₂ m⁻² s⁻¹; (G), micropropagated; (H), self-rooted) of micropropagated (MP) and self-rooted (SR) ‘Coratina’. Lowercase letters indicate significant differences before and after the irrigation; capital letters indicate differences among cultivars ($p < 0.05$).

Lastly, Pn showed significant improvements post-irrigation in both ‘Coratina’ MP and SR treatments, with a serious decrement at the end of the season. In fact, for MP, Pn was statistically higher from the beginning of the season until August (Figure 4G). In particular, a higher photosynthesis rate was recorded in the first measurement, which reduced in the middle of the summer, and the lowest value recorded was in DOY 196 (1.30 μmol CO₂ m⁻² s⁻¹). The trend was confirmed also in DOY 245–247 and, after irrigation, Pn performed almost double with respect to pre-irrigation (4.98 and 2.35 μmol CO₂ m⁻² s⁻¹). In SR, a similar, but less relevant, pattern was observed (Figure 4H). In fact, the highest Pn was observed in the first part of the season (5.80 and 7.30 μmol CO₂ m⁻² s⁻¹ before and after irrigation, respectively), with a continuous decrement through the summer. Also, in the last measurement, the trend was confirmed, with higher values in post-irrigation

($2.74 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Finally, ‘Coratina’ MP showed statistically higher Pn than SR in post-irrigation (6.20 vs. $3.25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for MP and SR, respectively).

3.3.3. ‘Frantoio’

LWP showed the same significant improvement post-irrigation for both propagation methods. In MP, it did not perform significantly better, except for in June’s measurements (Figure 5A). In fact, post-irrigation LWP was 16% higher than it was pre-irrigation (-2.57 and -3.05 MPa, respectively). Both pre- and post-irrigation values decreased throughout the season, showing no statistical differences. SR showed almost the same pattern but with different results (Figure 5B). Although the values were similar, in SR no differences were observed for the first measurement day, while statistical differences were observed at all other times. The highest difference was recorded in September, in which pre-irrigation performed the worst (-5.11 MPa) and post-irrigation achieved a 0.8 MPa increment (-4.33 MPa). Moreover, a slight statistical difference was observed in June between the two irrigation methods, with a higher LWP for MP (-2.81 MPa). The gs in ‘Frantoio’ demonstrated significant differences between pre- and post-irrigation conditions. MP showed higher gs values for post-irrigation in mid-summer (Figure 4C). In particular, at DOY 196–198, significantly higher gs was observed post-irrigation ($0.067 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) compared to pre-irrigation ($0.017 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). At the end of the season, no differences were observed. SR showed a different pattern (Figure 4D). Even if post-irrigation showed no statistical difference in the first measurement, differences were observed during the rest of the season, with the most relevant difference at DOY 217–219 (0.020 and 0.043 before and after irrigation $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Finally, the E increased significantly post-irrigation in both MP and SR, mostly in the middle of the season. MP showed differences in mid-summer measurements, with the most relevant variation at DOY 196–198 (2.99 and $1.11 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in post- and pre-irrigation, respectively) (Figure 5E). SR showed a different pattern, with constantly lower transpiration rates from the beginning of the season until the end (Figure 5F). Specifically, post-irrigation values were always statistically higher, with the highest E in July ($2.77 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), except for the first measurement. There were decreasing statistical differences until the last measurement (1.32 and $1.96 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in pre- and post-irrigation, respectively).

Pn exhibited significant increases post-irrigation in both ‘Frantoio’ MP and SR treatments in almost all seasons. MP showed higher Pn in all measurements, except for September (Figure 5G). In fact, even if irrigation played a relevant role in all three measurements, with the highest value in DOY 163 ($7.88 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), Pn showed no statistical difference in the last measurement (3.40 and $2.64 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ before and after irrigation, respectively). In the SR treatment, significant differences were observed for all the DOYs (Figure 5H). Pn was statistically higher in all seasons, with the biggest difference being in June (4.20 and $7.87 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in pre- and post-irrigation, respectively). Lastly, MP showed higher and statistically different values in the first measurement (5.71 and $4.20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for SR and MP, respectively).

3.3.4. ‘Urano’

LWP values were generally less negative post-irrigation, indicating improved water status. In MP, trees performed statistically better at the beginning and end of the season (Figure 6A). In fact, the highest values were observed in June (-2.67 MPa) and in September (-3.84 MPa), significantly higher than pre-irrigation (-3.30 and -4.75 MPa in DOYs 161 and 245, respectively). Different behavior was observed for SR (Figure 6B). Significant improvements were noted in July and September, with the highest LWP at DOY 198 (-3.03 MPa). Furthermore, statistical differences were observed between the two propagation methods post-irrigation, with higher potentials recorded in SR (-3.03 and -3.39 MPa for DOYs 198 and 217, respectively) than for MP (-3.61 and -4.20 MPa for DOYs 198 and 217, respectively).

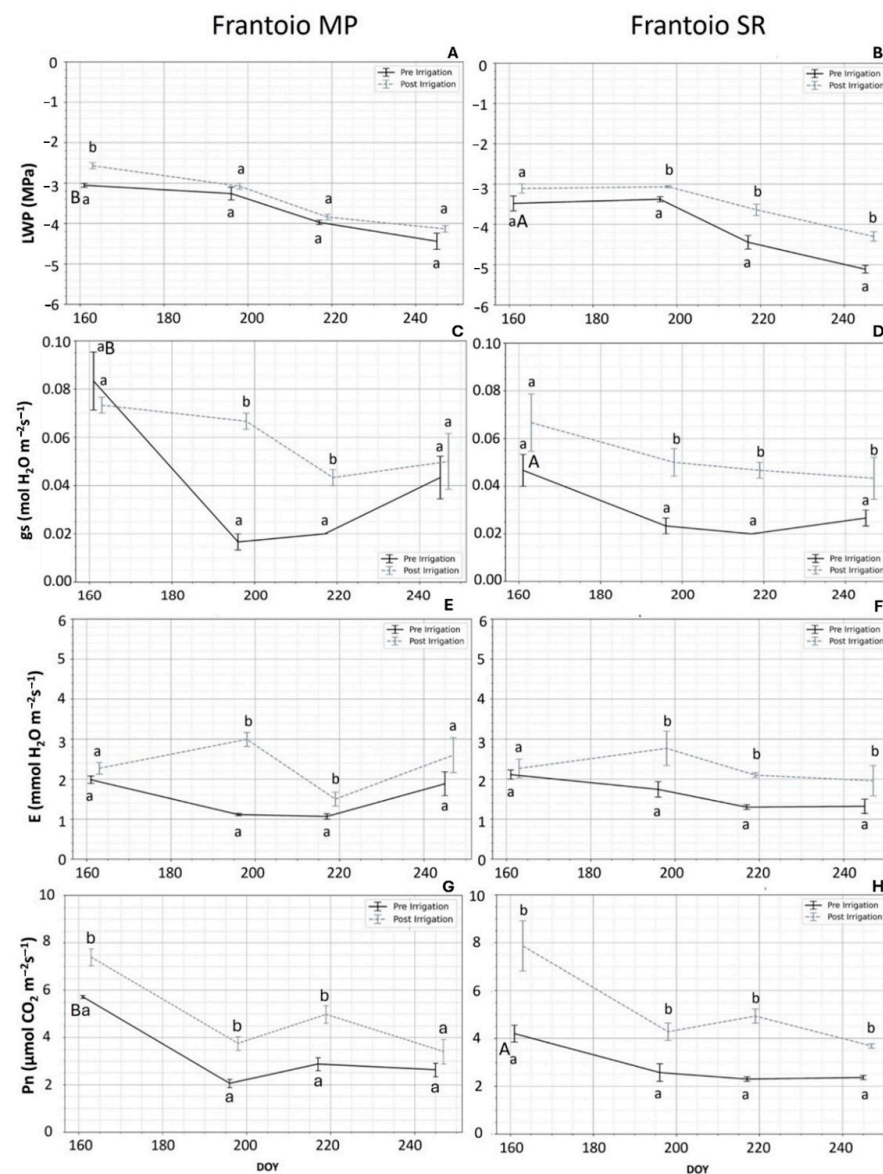


Figure 5. Mean and standard error of leaf water potential (LWP; MPa; (A), micropropagated; (B), self-rooted), stomatal conductance (gs; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$, (C), micropropagated; (D), self-rooted), transpiration rate (E; $\text{mmol HO m}^{-2} \text{s}^{-1}$; (E), micropropagated; (F), self-rooted), and net photosynthetic rate (Pn; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$; (G), micropropagated; (H), self-rooted) of micropropagated (MP) and self-rooted (SR) ‘Frantoio’. Lowercase letters indicate significant differences before and after irrigation; capital letters indicate differences among cultivars ($p < 0.05$).

Different gs patterns for Urano MP and SR. For MP, even though no differences were observed at the beginning and end of the season, significantly higher values were recorded post-irrigation in July and August (Figure 6C). In particular, at DOY 196–198, a higher than double value was observed post-irrigation (0.023 and $0.053 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ before and after irrigation, respectively), maintaining this trend in August also (0.020 and $0.040 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ in pre- and post-irrigation, respectively). In the SR-propagated trees, a different response to irrigation was recorded (Figure 6D): no difference was observed for almost all the season, except for August, in which post-irrigation showed a relevant recovery (0.040 and $0.050 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ for pre- and post-irrigation, respectively). Lastly, statistical differences were also observed in mid-summer between the two propagation methods. In particular, in July, Urano SR performed better pre-irrigation (0.037 vs. $0.023 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ for SR and MP, respectively) but worse post-irrigation (0.037 vs.

0.053 mol H₂O m⁻² s⁻¹ for SR and MP, respectively). Alternatively, in August, Urano SR showed higher stomatal conductance in both pre- and post-irrigation (0.020 and 0.040 mol H₂O m⁻² s⁻¹ for MP and SR pre-irrigation, and 0.050 and 0.040 mol H₂O m⁻² s⁻¹ for MP and SR post-irrigation, respectively). Also, E showed different recovery behavior between the two propagation methods. MP values were significantly improved with the irrigation (Figure 6E). While no differences were observed in June, a steady and significantly higher E was recorded from July to September. In particular, in July post-irrigation showed the highest recovery (2.90 vs. 1.49 mmol H₂O m⁻² s⁻¹ for post- and pre-irrigation, respectively). In the SR treatment, Urano showed a peculiar response to irrigation (Figure 6F). E was higher at the beginning of the season, showing higher values pre-irrigation than post (3.06 and 2.10 mmol H₂O m⁻² s⁻¹, respectively), and decreasing throughout the summer. In September, while pre-irrigation values remained under 2 mmol H₂O m⁻² s⁻¹, post-irrigation showed significantly higher E (2.33 mmol H₂O m⁻² s⁻¹). Moreover, a statistical difference between the two propagation methods was observed only in July, in which a better recovery was observed in MP (2.90 mmol H₂O m⁻² s⁻¹) compared to in SR (1.61 mmol H₂O m⁻² s⁻¹).

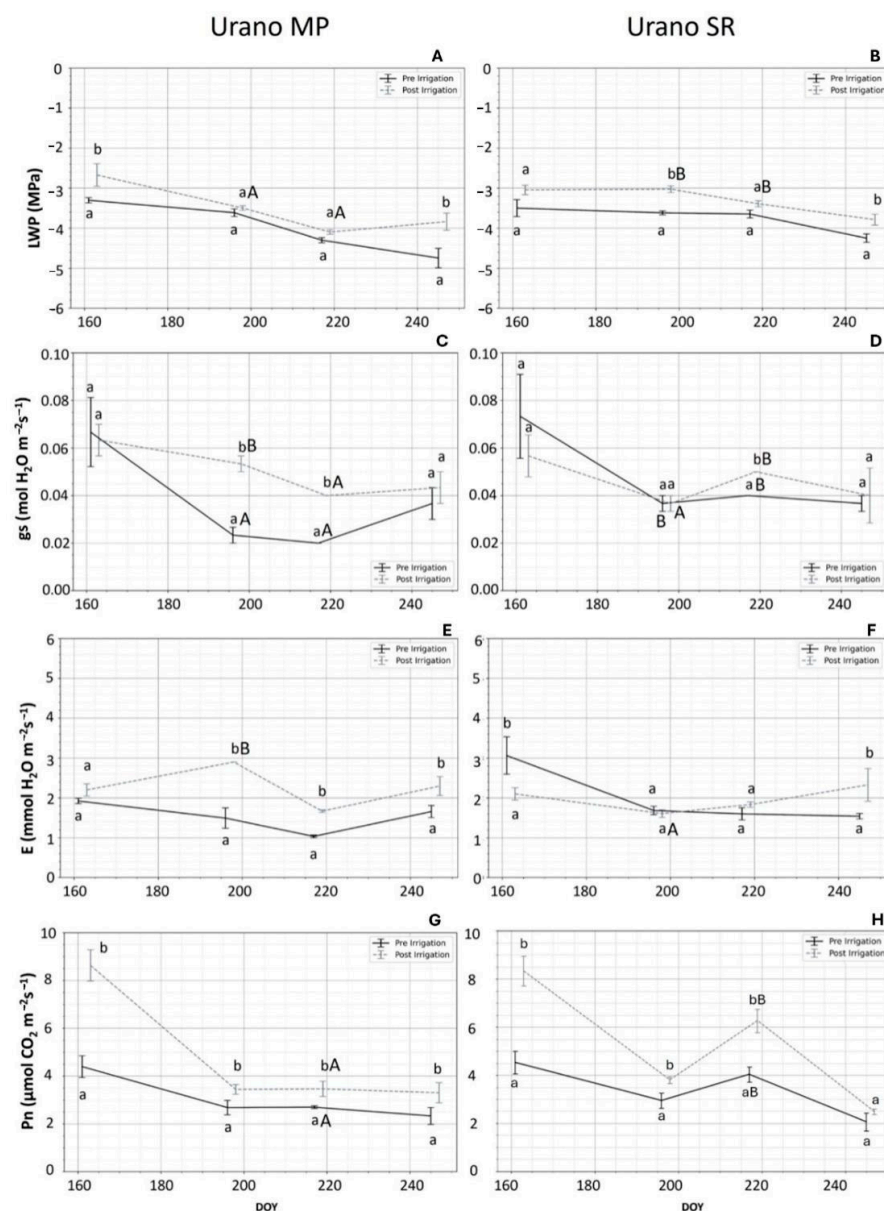


Figure 6. Mean and standard error of leaf water potential (LWP; MPa; (A), micropropagated; (B), self-rooted), stomatal conductance (gs; mmol H₂O m⁻² s⁻¹, (C), micropropagated; (D), self-rooted),

transpiration rate (E; $\text{mmol HO m}^{-2} \text{s}^{-1}$; (E), micropropagated; (F), self-rooted), and net photosynthetic rate (Pn; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$; (G), micropropagated; (H), self-rooted) of micropropagated (MP) and self-rooted (SR) ‘Urano’. Lowercase letters indicate significant differences before and after irrigation; capital letters indicate differences among cultivars ($p < 0.05$).

Pn performed differently between MP and SR. In MP, steadily higher values were recorded post-irrigation (Figure 6G). In particular, the highest recovery was observed in June (8.63 and $4.40 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ post- and pre-irrigation, respectively). During the rest of the summer, Pn decreased and reached its lowest values in September (2.33 and $3.30 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ for pre and post-irrigation, respectively). SR showed different behavior (Figure 6H). While trees showed an identical performance in the first part of the season, with the highest value recorded in June (8.33 and $4.53 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ before and after irrigation) and July (2.94 and $3.80 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), higher rates were observed in August: SR showed statistically higher values both pre-irrigation (4.03 and $2.70 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ for SR and MP, respectively) and post-irrigation (6.27 and $3.47 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ for SR and MP, respectively). In September, no statistical difference was observed either between pre- and post-irrigation, or between propagation methods.

3.4. Correlations

Pearson correlation showed weak and non-significant correlations across all parameters for ‘Arbequina’ for both MP and SR (Table 2). This suggested that stomatal conductance, net photosynthetic rate, and leaf water potential were not strongly interrelated, regardless of the propagation method.

Table 2. Pearson correlation between leaf water potential (LWP), stomatal conductance (gs), and net photosynthetic rate (Pn) of the four micropropagated (MP) and self-rooted (SR) cultivars considered in the study. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Cultivar	Propagation	gs vs. Pn	gs vs. LWP	E vs. LWP
‘Arbequina’	Micropropagated	0.38	0.20	0.31
	Self-rooted	0.33	0.25	0.26
‘Coratina’	Micropropagated	0.22	0.20	0.34
	Self-rooted	0.63 ***	0.66 ***	0.56 **
‘Frantoio’	Micropropagated	0.73 ***	0.44 *	0.18
	Self-rooted	0.87 ***	0.53 **	0.64 ***
‘Urano’	Micropropagated	0.62 **	0.54 **	0.42 *
	Self-rooted	0.45 *	0.21	0.12

The situation changed significantly for ‘Coratina’. In MP, the correlations between the parameters were weak and non-significant. However, in SR, the correlations became strong and highly significant. Specifically, the correlations between gs and Pn (0.63 ***), gs and LWP (0.66 ***), and E and LWP (0.56 **) indicated a strong interdependence among these parameters, suggesting a more pronounced mutual influence. For ‘Frantoio’, the correlations were significant in both conditions. In MP, there was a strong correlation between gs and Pn (0.73 ***), a moderate correlation between gs and LWP (0.44 *), and a weak, non-significant correlation between E and LWP (0.18). In SR, the correlations were even stronger: gs vs. Pn (0.87 ***), gs vs. LWP (0.53 **), and E vs. LWP (0.64 ***). This indicated that, for ‘Frantoio’, the three parameters were strongly interrelated. Finally, for ‘Urano’, the correlations were significant, primarily in MP conditions. The correlations between gs and Pn (0.62 **), gs and LWP (0.54 **), and E and LWP (0.42 *) were all significant. However, in SR, only the correlation between gs and Pn (0.45 *) was significant, while the other correlations were weak and non-significant. This suggested that for ‘Urano’, the propagation method affected the interdependence among these parameters.

4. Discussion

4.1. 'Arbequina'

The observed differences in g_s between 'Arbequina' MP and SR trees underscore the impact of irrigation on plant physiology. LWP improvements post-irrigation indicate an overall enhanced water status in both propagation methods, with SR showing a more stable and less reactive water status [21,67]. This stability suggests that SR may have a more extensive root network, enabling better access to water and reducing the immediate impact of irrigation fluctuations. In contrast, the more significant changes in LWP in MP highlight their sensitivity to soil moisture variations, possibly due to a less developed root system. Previous reports indicated that LWP values for 'Arbequina' could vary from -0.9 to -6.5 MPa, with fewer negative values indicating better water status, which supports the observed improvements post-irrigation. In particular, Diaz-Espejo [21] observed that, with less than -4 MPa, 'Arbequina' starts to cavitate. P_n enhancements post-irrigation, particularly in SR, can be attributed to their better water status and increased stomatal conductance, facilitating greater CO_2 uptake for photosynthesis. SR consistently exhibited higher g_s values post-irrigation, indicating their superior ability to maintain open stomata for gas exchange under improved water availability. This suggests that probably SR cultivars have a more efficient root system, enhancing their capacity for water uptake and utilization. The varying responses in MP, with less pronounced increases in g_s , point to potential differences in root structure or water transport mechanisms that may limit their responsiveness to irrigation compared to SR. Previous studies reported g_s values for 'Arbequina', reaching up to $0.4 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ under non-stress conditions, which aligns with the higher post-irrigation values observed in this study [16,22,23]. E also reflected the enhanced water uptake capabilities of SR, which maintained higher transpiration rates post-irrigation [17,19,20]. This efficiency in water transport likely contributes to the overall better physiological performance of SR cultivars, allowing them to sustain higher transpiration, even under fluctuating environmental conditions. The less consistent increases in E observed in MP further emphasize the potential limitations in their water transport systems, affecting their overall ability to regulate internal water status effectively. Historical data showed leaf transpiration rates for 'Arbequina' ranging from 0.01 to $12 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, with higher rates typically observed early in the season and decreasing thereafter, which is consistent with the observed post-irrigation increments in SR. The consistent improvements in P_n in SR across multiple sampling dates indicate their superior efficiency in converting available water into biomass. Conversely, the delayed or reduced photosynthetic response in MP suggests inherent physiological differences that may limit their photosynthetic capacity under similar irrigation conditions. Previous data for 'Arbequina' showed P_n values ranging from 17 to $32 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ around DOY 180, with higher rates early and late in the season, aligning with the enhanced P_n observed post-irrigation in SR [16,19,25]. Overall, the differences observed between the propagation methods highlight the superior physiological adaptability of self-rooted 'Arbequina' olive trees to irrigation. These trees demonstrate more robust water uptake and utilization mechanisms, leading to better overall performance under irrigated conditions. In contrast, micropropagated trees, while capable of achieving similar physiological states, exhibit more variability in their responses, likely due to differences in root development and water transport efficiency.

4.2. 'Coratina'

The increase in post-irrigation g_s for both 'Coratina' MP and SR underscored the positive impact of irrigation on plant physiology. LWP values were generally less negative post-irrigation, indicating an overall improvement in plant water status. MP showed consistently higher LWP values post-irrigation, with significant differences observed mid-season. This suggests that micropropagated trees benefit significantly from irrigation, improving their water status, and potentially their overall health. SR followed a similar trend, with notable improvements mid-season. Historical LWP data for 'Coratina' ranged from -0.4 to -6.45 MPa, with less negative values reflecting better water status,

which is consistent with the improvements observed post-irrigation [13,14,20,68]. In MP, g_s values significantly increased mid-season and maintained higher levels post-irrigation, indicating a strong response to improved water availability. SR showed similar significant increases from mid-season to the end of the season, reflecting their enhanced capacity for gas exchange under irrigated conditions. Previous studies reported g_s values for ‘Coratina’ ranging from 0.01 to 0.39 mol H₂O m⁻² s⁻¹, indicating variability based on environmental conditions and irrigation status [13,14,20]. E significantly increased in post-irrigation for both ‘Coratina’ MP and SR, demonstrating the critical role of water availability in regulating plant water loss. MP cultivars consistently showed higher E values post-irrigation throughout the season, highlighting their ability to maintain higher transpiration rates under improved water conditions. SR exhibited significant increases in E , particularly mid-season, suggesting that their root systems effectively support water uptake and transpiration. Historical data indicated that E values for ‘Coratina’ ranged from 2.4 to 15 mmol H₂O m⁻² s⁻¹, with higher values observed during periods of sufficient water supply, which aligns with the post-irrigation increases observed in this study [19,20]. P_n improved significantly post-irrigation for both ‘Coratina’ MP and SR, although both experienced a decline towards the end of the season. MP showed higher P_n values post-irrigation from the beginning of the season until August, indicating a robust response to water availability that supports higher photosynthetic activity. SR exhibited a similar, though less pronounced, pattern, suggesting that while both methods benefit from irrigation, MP may have a more substantial photosynthetic response. Previous data for ‘Coratina’ reported P_n values ranging from 7 to 19 µmol CO₂ m⁻² s⁻¹ [19,69], with higher values observed under optimal water conditions, supporting the enhanced P_n observed post-irrigation in both propagation methods.

4.3. ‘Frantoio’

In ‘Frantoio’, g_s showed significant differences between pre- and post-irrigation conditions, particularly mid-season. LWP demonstrated significant improvements post-irrigation for both propagation methods. MP showed a notable improvement in June, reflecting a 16% increase post-irrigation. This indicates that irrigation substantially improves the water status of MP, particularly early in the season. For SR, significant differences were observed in all measurements except the first. The highest improvement was recorded in September, where post-irrigation LWP was markedly better than pre-irrigation. Additionally, MP exhibited slightly better water potential in June compared to SR, suggesting some variability in how each propagation method manages water status under irrigation. Observed data are in line with previous research, in which ‘Frantoio’ could also withstand very low potentials (−6, −8 MPa), working well at −2.5 MPa [24]. Previous studies showed that ‘Frantoio’ could restrict water loss through stomatal regulation under drought conditions [15,24,70]. However, cultivar-specific differences in water relations suggest that targeted irrigation strategies could be developed to optimize water use and maintain yield [15]. For MP, g_s values were significantly higher post-irrigation during mid-summer, suggesting a robust response to increased water availability. The most significant difference was observed at DOY 196–198, indicating that MP could better regulate their gas exchange under irrigation. In SR, significant differences in g_s were observed throughout the season, particularly at DOY 217–219. This pattern suggests that SR cultivars maintain a consistent ability to increase stomatal conductance in response to irrigation, though the initial measurement showed no significant difference. Previous studies reported g_s values for ‘Frantoio’ ranging from 0.02 to 0.4 mol H₂O m⁻² s⁻¹ under various conditions, which aligns with the observed post-irrigation increases in this study [18]. E exhibited significant increases post-irrigation for both ‘Frantoio’ MP and SR, especially in mid-summer. MP showed notable increases at DOY 196–198, highlighting their enhanced capacity to manage water loss when adequately irrigated. SR consistently had higher post-irrigation E values throughout the season, indicating their effective water transport system. Wide variations could be observed during the season. Proietti and Famiani [70] observed lower E during August instead of June.

Pn increased significantly post-irrigation for both 'Frantoio' MP and SR throughout most of the season. MP showed higher photosynthesis rates in all measurements except for September, indicating a sustained positive response to irrigation. The most significant improvement was observed in June, with a peak Pn value early in the season. In SR, significant differences were recorded for all DOYs, with the highest Pn observed in June. During drought stress, olive trees showed reduced growth and photosynthetic activity. However, they can maintain a slight net photosynthetic rate (about 10% of well-watered trees) at very low pre-dawn leaf water potentials by closing stomata to prevent excessive water loss [24]. Previous data for 'Frantoio' reported Pn values ranging from 4 to 20 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ [18], which aligns with the significant improvements observed in this study under post-irrigation conditions.

4.4. 'Urano'

The cv Urano is one of the least investigated of all studied cultivars, which prevents comparisons [71] between olive trees showing distinct physiological responses to irrigation and propagation. LWP improved post-irrigation for both MP and SR, indicating better water status. MP showed significant improvements at the beginning and end of the season, with the highest values recorded in June and September. This suggests that MP quickly benefitted from irrigation, maintaining better water status during critical growth periods. SR also demonstrated significant improvements mid-summer and in September, with higher LWP values post-irrigation compared to MP during these times. The differences between propagation methods post-irrigation, with SR showing better water potential, indicate that SR might have a more efficient water uptake and retention system under irrigated conditions. Specific LWP data for 'Urano' were not provided; however, improvements are consistent with expected physiological responses to irrigation. For 'Urano' MP, gs exhibited significant increases post-irrigation during July and August. This suggests that MP are highly responsive to irrigation during mid-summer, effectively utilizing the available water to maintain higher gs values. The most notable increase was at DOY 196–198, with gs values more than doubling post-irrigation. Conversely, 'Urano' SR displayed significant differences primarily in August, indicating a delayed response to irrigation compared to MP. Additionally, significant differences between the two propagation methods were observed during mid-summer, with SR performing better in pre-irrigation conditions and MP outperforming SR post-irrigation. Previous studies did not provide specific gs data for 'Urano', but similar trends in response to irrigation can be observed from other cultivars. E also showed distinct patterns between the propagation methods. MP had significantly higher E values post-irrigation from July to September, reflecting their ability to enhance water loss and gas exchange under irrigated conditions. The highest recovery was in July, highlighting the critical role of irrigation during peak summer stress. SR, however, exhibited higher E early in the season, even in pre-irrigation conditions, but showed a significant decline throughout the summer. By September, SR demonstrated a substantial recovery post-irrigation, though overall it was lower than MP. The statistical differences observed in July between the two methods suggest that MP cultivars benefit more from irrigation during this critical period. Historical data for 'Urano' E were not available, so no comparisons can be made. Pn displayed different trends between MP and SR. MP consistently showed higher Pn values post-irrigation, particularly in June, indicating a strong initial response to improved water availability. However, Pn values decreased throughout the summer, reaching the lowest in September. SR, on the other hand, showed significant improvements in Pn, post-irrigation in August, maintaining higher photosynthetic activity during the latter part of the season. This suggests that SR can sustain photosynthetic efficiency under prolonged irrigation conditions better than MP. Notably, in August, SR had higher Pn in both pre- and post-irrigation compared to MP, highlighting their superior adaptation to mid-summer conditions. Specific Pn data for 'Urano' were not available; however, the trends observed are consistent with those of other cultivars under similar conditions.

5. Conclusions

This study provided the first field evaluation data from an ecophysiological perspective on the seasonal patterns of recovery of four olive cultivars in a super high-density orchard of both self-rooted and micropropagated olive trees. The research highlighted significant differences between micropropagated (MP) and self-rooted (SR) trees, with SR consistently exhibiting higher g_s , E , and P_n post-irrigation compared to MP, which showed more variability likely due to differences in root development and water transport mechanisms.

‘Arbequina’, known for its water stress tolerance, maintained high net P_n under deficit conditions due to osmotic adjustment mechanisms, but MP trees exhibited significant variability in response to water stress, with reductions in P_n due to decreased g_s and photosynthesis. Moreover, SR had a better recovery response both for g_s and E with respect to MP. On the other hand, recovery for MP showed interesting responses only at the end of the season, showing lower relevance of the response to irrigation. For these reasons, a constant control of the ecophysiological state is strongly advised, considering its relevant effect in responding to the incoming stresses.

‘Coratina’ showed notable increases in g_s and E post-irrigation, reflecting its ability to manage gas exchange and water loss under improved water conditions. This cultivar exhibited a better response in the middle of the season, corresponding to mid-summer, in which the irrigation had the most effect, with less relevant differences at the end of the season. MP trees responded better in this period both for LWP and for P_n , while no relevant differences were observed in the other two parameters. These findings are useful to understand that the June–July period is the most important to improve trees status. A higher number of measurements in this period could give strong results, leading to the best cultivar production possible.

‘Frantoio’ demonstrated greater resilience, maintaining higher water potential and some photosynthetic activity under water stress, with strong interrelationships among g_s , P_n , and LWP. As for ‘Coratina’, this cultivar exhibited the most relevant recovery from the mid-summer on. In particular, DOYs 198 and 219 had the highest delta for P_n in the MP trees. Anticipating the stresses through a constant measurement of these parameters can avoid considerable losses, like in DOY 196, ensuring a better general status of the orchard. SR trees expressed a higher recovery relevance than MP ones. In this case, the utilization of this parameter to check other parameters’ status is interesting, so the use of only Scholander’s chamber can lead to success.

‘Urano’ exhibited distinct physiological responses, with MP trees highly responsive to mid-summer irrigation, maintaining higher g_s values, and SR trees showing significant P_n improvements post-irrigation, especially late in the season. ‘Urano’ also expressed the highest differences between the two PM. While MP trees had a better E and g_s in the first part of the summer, SR ones showed higher values for LWP, g_s , and P_n in the second part of the season. SR has shown better ecophysiological recovery than MP, mostly always in the middle of the summer.

This research underscores the importance of micropropagation on olive tree ecophysiological recovery. Understanding cultivar-specific responses to irrigation and recovery patterns allows growers to implement targeted irrigation strategies to enhance productivity and sustainability in super-high-density orchards. Future research should focus on long-term studies to assess the cumulative effects of different propagation methods on olive tree physiology and productivity. Investigating interactions between propagation methods and other farming practices, such as irrigation regimes and nutrient management strategies, would provide a comprehensive understanding of optimizing olive tree performance in SHD orchards as well. Future studies should address these limitations by including a broader range of environmental conditions, cultivars, and experimental designs over multiple growing seasons.

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