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Gas Exchanges and Stem Water Potential Define Stress Thresholds for Efficient Irrigation Management in Olive (*Olea europaea* L.)

Giulia Marino ^{1,2,*}, Tiziano Caruso ², Louise Ferguson ³  and Francesco Paolo Marra ² 

¹ Department of Land Air and Water Resources, 213, Veihmeyer Hall, University California Davis, One Shields Ave., Davis, CA 95616, USA

² Dipartimento Scienze Agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze Edificio 4 Ingresso H, 90128 Palermo, Italy; tiziano.caruso@unipa.it (T.C.); francescopaolo.marra@unipa.it (F.P.M.)

³ Department of Plant Sciences, Wickson Hall, University of California Davis, One Shields Avenue, Davis, CA 95616, USA; lferguson@ucdavis.edu

* Correspondence: giumarino@ucdavis.edu; Tel.: +1-530-304-4509

Received: 4 February 2018; Accepted: 15 March 2018; Published: 20 March 2018

Abstract: With climate change and decreased water supplies, interest in irrigation scheduling based on plant water status is increasing. Stem water potential (Ψ_{SWP}) thresholds for irrigation scheduling in olive have been proposed, however, a physiologically-based evaluation of their reliability is needed. A large dataset collected at variable environmental conditions, growing systems, and genotypes was used to characterize the relation between Ψ_{SWP} and gas exchanges for olive. Based on the effect of drought stress on the ecophysiological parameters monitored, we described three levels of stress: no stress (Ψ_{SWP} above about -2 MPa), where the high variability of stomatal conductance (g_s) suggests a tight stomatal control of water loss that limit Ψ_{SWP} drop, irrigation volumes applied to overcome this threshold had no effect on assimilation but reduced intrinsic water use efficiency (iWUE); moderate-stress (Ψ_{SWP} between about -2.0 and -3.5 MPa), where iWUE can be increased without damage to the photosynthetic apparatus of leaves; and high-stress (Ψ_{SWP} below about -3.5 MPa), where g_s dropped below $150 \text{ mmol m}^{-2} \text{ s}^{-1}$ and the intercellular CO_2 concentration increased proportionally, suggesting non-stomatal limitation to photosynthesis was operative. This study confirmed that olive Ψ_{SWP} should be maintained between -2 and -3.5 MPa for optimal irrigation efficiency and to avoid harmful water stress levels.

Keywords: assimilation; stomatal conductance; photosynthesis; water status; water use efficiency

1. Introduction

Olive (*Olea europaea* L.) is a drought resistant crop traditionally grown under rainfed condition in low density plantation. Recently, with the introduction of the super-high-density (SHD) system, characterized by very high planting density (1600–2000 trees per ha) and trees trained as hedgerows, olive orchard management has changed deeply. SHD orchards offer many advantages: they reach productivity very few years after planting, allow mechanization of harvesting and partial mechanization of pruning (tree topping) and a significant reduction in production costs [1–3]. However, increased planting density increases light interception [4], root competition [5], and plant sensitivity to drought [6]. This suggests irrigation parameters may also need to change. Irrigation is generally based on orchard water use (e.g., crop evapotranspiration, ET_c), but multiple studies have demonstrated this method, because it does not consider plant water status, may overestimate the irrigation required for optimal yield [7–9] and reduce orchard water use efficiency.

More efficient, plant-based irrigation scheduling would be of great benefit as climate change advances and water supplies dwindle [10,11]. Plant-based irrigation methods recognize the tree as the best indicator of its water status, and allow growers tailoring water applications to actual tree's needs and status, thus potentially reducing the non-beneficial water uses and ensuring a precise stress control.

Among the current water status measurements available, stem water potential (Ψ_{SWP}) measured at midday, is the most reliable, and most widely studied [7,12–15]. It is used as reference of water status to calibrate new methodologies, define waters stress levels and manage deficit irrigation methodologies. However, the reported values of Ψ_{SWP} are not consistent. This lack of consistent results is probably a function of environmental variability among orchards, primarily evaporative demand [16] and the different drought resistance mechanisms of the different olive genotypes [6,17].

When stomatal conductance is used as stress indicator common pattern on the response of photosynthesis to stress intensity are found [16].

Three stages of photosynthesis inhibition as a function of water stress intensity are reported [18–21].

Under mild-to-moderate drought-stress, a parallel decline in A_n and g_s is observed [12]. In this first stage stomata close to prevent water loss, reducing stomatal conductance (g_s), photosynthetic rate (A_n) [22,23], and shoot growth and fruit production [24]. Therefore, intrinsic water use efficiency (iWUE) increases with stress.

More severe water deficits (stage II and III) strongly and directly affect net photosynthesis [18,25–28] and the tree's ability to recover from stress [29,30].

In stage II C_i and g_s are directly correlated showing that stomatal limitation are still predominant while in stage III C_i increases as stomata close suggesting metabolic impairments to the photosynthetic system are predominant. In this last stage, iWUE decreases.

Collectively, the results demonstrate that a physiological characterization of stress levels is needed for a more sensitive plant based deficit irrigation scheduling, with the objective of increasing WUE while maintaining yield.

In this study, we aim to characterize the relationship between midday stem water potential and gas exchange for olive at variable crop conditions. We used a large dataset from two different experiments carried out in recent years [7,17].

The variability of environmental conditions (two different locations in two different years), genotypes ('Arbequina', 'Nocellara del Belice', and 'Olivo di Mandanici') and planting design and tree density (super high density, high density, and low density) provide sufficient wide dataset to smooth out variability in physiological behavior generally associated to difference in experimental conditions [31].

The different mechanisms involved in stomatal and non-stomatal control of photosynthesis at the different stress levels are investigated and used to define Ψ_{SWP} thresholds for irrigation of olive orchards that satisfy good physiologically-based criteria for a sustainable and efficient irrigation management.

Overall aim was to provide practical recommendation to improve irrigation management and avoid harmful stress while maintaining orchard productivity.

2. Materials and Methods

Data were collected during two different experiments. The first experiment was carried out in 2009 season in the same SHD orchard (cultivar 'Arbequina', 1905 trees/ha) where Marra et al. [7] made their experiment.

The orchard was planted in 2004 on a private farm located in Southern Italy (37°48'0" N, 12°26'0" E, 12 m altitude). The climate of the area and the experimental soil have been described in Marra et al. [7].

Five different irrigation treatments were imposed, corresponding to 16%, 21%, 41%, 62% and 83% of Crop Water Requirement (CWR). The FAO CROPWAT model for deficit irrigation scheduling was used to calculate CWR starting from the ETc and rain data.

ETc was established a priori using FAO procedure published as FAO Irrigation and Drainage Paper No. 56 [32]. In particular, ETc was calculated as $ETc = ETo \times Kc \times Kr$, where ETo (30-year average reference evapotranspiration) was estimated using the Penman–Monteith equation and environmental data provided by a public weather station (SIAS, Servizio Informativo Agrometeorologico Siciliano), located in the proximity of the orchard; Kc is crop coefficient obtained from literature [32–36] and varied from 0.50 to 0.75, depending on the phenology of the trees; Kr is a coefficient related to the percentage of ground covered by the crop and resulted 0.58 based on measurements of canopy volume and subsequent calculations of the percentage of ground area coverage (42%).

Details on the irrigation system and orchard practices were also given in Marra et al. [7].

A randomized block design was used with 5 blocks of 45 trees each (9 trees per treatment), distributed between three adjacent rows; within each block one tree per irrigation treatment was randomly selected in the central row and subsequently monitored. Stem water potential (Ψ_{SWP}) and gas exchanges were measured on the 11 August, when the trees are more resistant to drought, and 1 September, when the trees are sensitive to drought [37].

The second experiment was carried out in 2014, in two adjoining olive orchards located near Sciacca (37°32' N, 150 m above sea level) in southwest Sicily (Italy), the same area where Marino et al. [15] conducted their experiment. A more detailed description of the climate and the soil is reported in Marino et al. [6].

Out of the two adjoining orchards, one was a traditional 10-year-old widely spaced (200 trees/ha) orchard (cv. 'Nocellara del Belice'). Trees were trained as vase shape and rain-fed all over the season.

The adjoining orchard was a hedgerow trained high density (HD, 1000 trees/ha) 3-year-old orchard. Two genotypes ('Nocellara del Belice' and 'Olivio di Mandanici'), characterized by different vigor and productive potential [38] were monitored for their response to short term water deficit. The irrigation was supplied from June, every 7–10 days. At the beginning of July, irrigation was stopped for a number of days until the midday Ψ_{SWP} decreased below -2.5 MPa [7,26]. A re-watering period followed. Detail about orchard characteristics, experimental design and irrigation management are reported in Marino et al. [6]. Stem water potential and gas exchange were monitored every 10 days, from June until October.

Stem water potential was measured at midday using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Fully-exposed shoots of the current growth season with five or six expanded leaves were covered with plastic envelopes and aluminum reflective foil at least 1 h before measurement in order to reduce leaf transpiration [39] and equilibrate stem water potential with branch water potential. On the same days when the Ψ_{SWP} was measured, we conducted a series of leaf gas exchange measurements on fully expanded leaves, using a CIRAS-2 (PP system®) portable gas exchange system (CO_2 and H_2O) connected to a gas exchange chamber (Parkinson Leaf Cuvette). The system measured principal eco-physiological parameters, such as light-saturated net CO_2 assimilation (A_n , $\mu mol\ m^{-2}\ s^{-1}$), stomatal conductance (g_s , $mmol\ m^{-2}\ s^{-1}$), atmospheric pressure, air and leaf temperature, and air CO_2 and intercellular CO_2 concentration (C_i) [40,41]. Intrinsic water use efficiency (iWUE, $mmol\ mol^{-1}$) was calculated as the relationship between A_n and g_s .

A commercial software package (TableCurve 2D; SYSTAT Software Inc., Chicago, IL, USA) was used to find the best-fit and the function parameters of the relationship between the principal eco-physiological variables.

Statistical analysis of the data (GLM) was carried out using the Systat (SYSTAT Software Inc., Chicago, IL, USA); significance was set at $p \leq 0.05$.

3. Results

As shown in Figure 1, A_n and g_s were well correlated with midday Ψ_{SWP} (r^2 of 0.61 and 0.51, respectively).

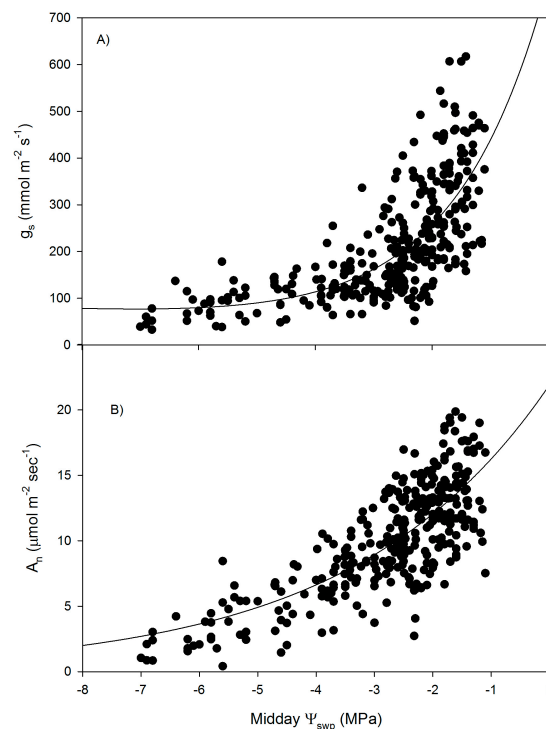


Figure 1. Relationship between (A) stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and (B) net photosynthesis (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) relative to the stem water potential (Ψ_{SWP} , MPa) measured at midday. The best fit relationship for the entire g_s data pool was performed using a double exponential function: $y = 39 \times e^{(-0.08x)} + 744 \times e^{(0.63x)}$; $r^2 = 0.51$; $p < 0.0001$; the best fit relationship for the entire A_n data pool was performed using a double exponential function: $y = 3.1 \times e^{(0.29x)} + 18.9 \times e^{(0.30x)}$; $r^2 = 0.61$, $p < 0.0001$.

The Ψ_{SWP} ranged from minimum values of -7 MPa to maximum values of -1 MPa. g_s ranged from $30 \text{ mmol m}^{-2} \text{s}^{-1}$ at Ψ_{SWP} lower than -6 MPa up to $600 \text{ mmol m}^{-2} \text{s}^{-1}$ at Ψ_{SWP} higher than -2 MPa; for the same Ψ_{SWP} range, A_n varied from maximum values of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ to minimum values of $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Data were fitted against a double exponential function. The high variability of the response of g_s and A_n relative to Ψ_{SWP} at different degree of stress supports this approach of curve fitting and distinguished three regions of Ψ_{SWP} .

For Ψ_{SWP} under the threshold of approximately -3.5 MPa, gas exchange maintained relatively constant and low rates, and Ψ_{SWP} slightly affected gas exchange. For instance, each unit increase in Ψ_{SWP} increased g_s by $25 \text{ mmol m}^{-2} \text{s}^{-1}$ (8% of total g_s increase) and A_n of $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ (12% of total A_n increase).

When Ψ_{SWP} increased, its relative influence on gas exchange was higher. One unit increase in Ψ_{SWP} within the range of -3.5 to -2 MPa increased g_s by $88 \text{ mmol m}^{-2} \text{s}^{-1}$ (25% of total g_s increase) and A_n by $2.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ (18% total A_n increase).

Above the threshold of -2 MPa, for each unit increase in Ψ_{SWP} , g_s increased by $137 \text{ mmol m}^{-2} \text{s}^{-1}$ (40% of total g_s increase) while A_n increased by 5 (35% of total A_n increase). Higher dispersion of data points around the curve was observed at increasing Ψ_{SWP} , suggesting the superimposition of other factor at low stress levels. This was particularly clear at Ψ_{SWP} above -2 MPa where, for the same Ψ_{SWP} value, g_s varied from 98 to $547 \text{ mmol m}^{-2} \text{s}^{-1}$.

Sub-stomatal CO_2 concentration (C_i) curvilinearly decreased, from approx. $250 \mu\text{mol mol}^{-1}$ to minimum values of $160 \mu\text{mol mol}^{-1}$, as g_s decreased from 600 to $100 \text{ mmol m}^{-2} \text{s}^{-1}$, a (Figure 2). For g_s under the threshold of $150 \text{ mmol m}^{-2} \text{s}^{-1}$ a deviation from the curve was observed and C_i started to increase for a group of datapoints. These datapoints, characterized by g_s lower than $150 \text{ mmol m}^{-2} \text{s}^{-1}$

and C_i higher than $220 \mu\text{mol mol}^{-1}$ (average of all the data), were plotted separately and were all characterized by Ψ_{SWP} lower than -3.5 MPa .

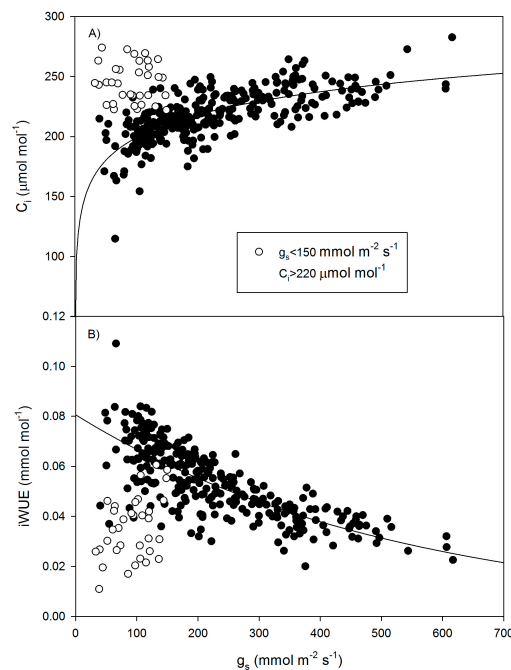


Figure 2. Relationship between (A) the sub stomatal CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$) and (B) intrinsic water use efficiency (iWUE, mmol mol^{-1}) relative to stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$). Data characterized by $g_s < 150 \text{ mmol m}^{-2} \text{s}^{-1}$ and $C_i > 220 \mu\text{mol mol}^{-1}$ (average of the population) were plotted separately. The best fit relationship for C_i was performed using a logarithmic function: $y = 71.8 + 26.7 \times \ln(\text{abs}(x))$; $r^2 = 0.52$. The best fit relationship for iWUE was performed using an exponential function: $y = 0.80 \times e^{(-0.019x)}$; $r^2 = 0.58$.

The relation between g_s and A_n was positive and exponential (Figure 3). To better visualize the effect of the stress on stomatal control of photosynthesis, we separately plotted data in three groups based on the analysis of the previous curves (Figures 1 and 2): data characterized by Ψ_{SWP} lower than -3.5 MPa , data characterized by Ψ_{SWP} between -3.5 and -2 MPa and data characterized by Ψ_{SWP} higher than -2 MPa .

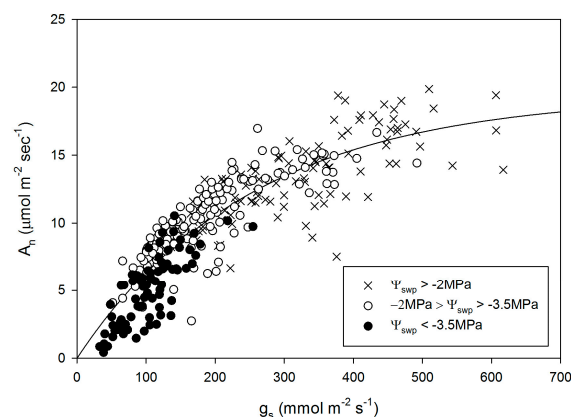


Figure 3. Relationship between net photosynthesis (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$). The best fit relationship, for the entire data pool, was obtained using an exponential function: $y = 19.4 \times (1 - e^{(-0.0039x)})$; $r^2 = 0.80$; $p < 0.0001$.

For Ψ_{SWP} below -3.5 MPa, g_s values were below approx. $150 \text{ mmol m}^{-2} \text{ s}^{-1}$ and the A_n increased sharply and linearly from 0 to $8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ as stomata progressively opened. For Ψ_{SWP} ranging from -2 to -3.5 MPa, the relation between g_s and A_n was curvilinear. A_n increased from 8 to $15 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ and g_s from 150 to $350 \text{ mmol m}^{-2} \text{ s}^{-1}$. The points characterized by a Ψ_{SWP} above -2 MPa were in the asymptotic part of the curve highlighting a wide variability in g_s (ranging from 350 to $600 \text{ mmol m}^{-2} \text{ s}^{-1}$) corresponding to a low variability in A_n (from 15 to $17 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$).

Intrinsic water use efficiency (iWUE) had the highest values (median of $0.06 \text{ mmol mol}^{-1}$) in plants characterized by Ψ_{SWP} between -3.5 and -2 MPa. Lower iWUE values (about $0.04 \text{ mmol mol}^{-1}$) were observed when Ψ_{SWP} decreased below -3.5 MPa or increased above -2 MPa (Figure 4).

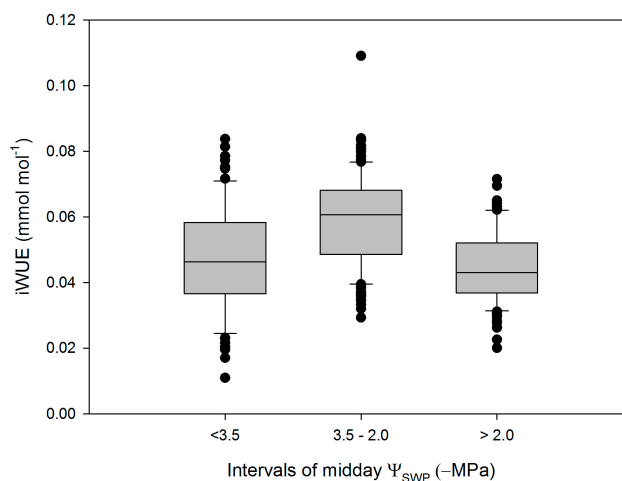


Figure 4. Intrinsic water use efficiency (iWUE, mmol mol^{-1}) for the selected midday stem water potential (Ψ_{SWP} , MPa) intervals.

4. Discussion

The non-linear relation observed by analyzing the pooled data of Ψ_{SWP} and g_s clearly suggests that the mechanisms involved in stomatal control vary according to the stress level [42].

Multiple studies have reported on the threshold for this relationship [23,26,43]. Generally, the best fit, in accordance to our study, is positive and exponential [44–46] but also linear [23] and logistic regressions were observed [26]. The main reason for this discrepancy can be the limited variability of Ψ_{SWP} values. Ψ_{SWP} data set of this experiment widely ranged, from -1 to -7 MPa, and g_s increased from 20 to more than $600 \text{ mmol m}^{-2} \text{ s}^{-1}$. If only the data points characterized by a Ψ_{SWP} above -3.5 MPa are used, a linear and weaker regression is observed also in this work (data not shown); if only data above -2 MPa are analyzed no relationship between Ψ_{SWP} and g_s is observed.

The marked variability in g_s (ranging from 600 to $250 \text{ mmol m}^{-2} \text{ s}^{-1}$) detected in this work for Ψ_{SWP} above -2 MPa, suggest that a $\Psi_{\text{SWP}}-g_s$ model at very low level of stress is not reliable.

Such high variability of g_s is probably due to the superimposition of environmental factors affecting stomatal behavior in well-irrigated trees [42]. This has been previously demonstrated for olive by Moriana et al. [12], reporting vapor pressure deficit to affect the relationship between Ψ_{SWP} and g_s only at low to moderate stress while no effect was observed at high stress. Oscillation of stomatal conductance that occur in olive, mainly in non-stressed plants [47,48], may contribute to increased variability in measured g_s values at low to no-stress conditions.

As the stress intensified ($-3.5 \text{ MPa} < \Psi_{\text{SWP}} < -2 \text{ MPa}$ in this work), stomata progressively closed suggesting hydraulic feedback largely controls the mechanism [42]. Mildly stressed olive trees restrict excessive water loss and prevent an excessive drop in Ψ_{SWP} by modulating stomatal closure [49], which is the earliest response to drought, and the major limitation to photosynthesis at mild to moderate drought [27].

At very high levels of stress ($\Psi_{SWP} < -3.5$ MPa) the small variation of both g_s and A_n in response to Ψ_{SWP} confirm that water deficit override the effect of other parameters on olive physiology and on stomatal control of gas exchange [50].

A double exponential equation was used because allowed to better distinguish the different mechanisms involved in stomatal response to drought [42] with respect to a single exponential curve. Variation in g_s affected the assimilation rate differently depending on the drought level as demonstrated by the analysis of the curve in Figure 3. This allows definition of different regions in this relationship, characterized by the different effects of drought on photosynthesis.

The first region is represented by the curvilinear and slight decrease in A_n for g_s decreasing from maximum values to $250 \text{ mmol m}^{-2} \text{ s}^{-1}$. Very high maximum values of g_s were observed reaching up to $600 \text{ mmol m}^{-2} \text{ s}^{-1}$. These values are higher than what reported by different authors [31,51,52]. However, similarly to what reported by Fernandez et al. [32], the increase in transpiration did not correspond to an increase in A_n that showed maximum value of $20 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$.

In this range of values, a decrease in g_s corresponded to a parallel decline in the sub-stomatal CO_2 concentration (Figure 2), which directly affected the photosynthetic rate.

Considering that plants were characterized by Ψ_{SWP} values above -3.5 MPa, this suggested that at low to mild stress levels, stomatal limitations to photosynthesis were dominant. At this level of stress, consistent with earlier reports [49,53,54], progressive drought increased iWUE (Figures 2B and 4).

Non-stomatal factors were predominant at higher levels of stress. For example, as Ψ_{SWP} dropped to -3.5 MPa and g_s dropped below $150 \text{ mmol m}^{-2} \text{ s}^{-1}$, C_i increased proportionally (Figure 3), reflecting the impaired photosynthetic metabolism in these plants, and, consequentially, iWUE decreased (Figures 2A and 4). For instance, a steeper slope of the function A_n for g_s , observed for values lower than $150 \text{ mmol m}^{-2} \text{ s}^{-1}$, reflects the superimposition of non-stomatal factors affecting photosynthesis (Figure 2). Other authors found a similar pattern with C_i , initially declining with increasing stress and then increasing as drought stress became more severe [55]. Other studies confirm the possibility of using C_i as an indicator of the stomatal or non-stomatal limitations to photosynthesis [27,56,57]. In an experiment conducted on nine different conifer species, Brodribb [58] observed that a rapid increase in C_i values at certain levels of water stress was accompanied by a rapid loss of fluorescence from PSII, suggesting damage to the photosynthetic apparatus of plants under drought beyond the minimum C_i , also called the C_i inflexion point by Flexas et al. [20]. Finally, analysis with data from literature, using g_s as a reference [19,25], demonstrated that the point at which C_i starts to increase is accompanied by a steep reduction in the net photosynthetic rate (A_n , reduced by 70%), in the CO_2 -saturated rate of photosynthesis (A_{sat} , reduced by 50%), in the apparent carboxylation efficiency (ϵ , reduced by 50%) and in the rate of light-saturated electron transport (ETR, reduced by 40%).

5. Conclusions

The results of this experiment provide physiological data to support the plant based irrigation schedule of an olive orchard designed to increase water use efficiency.

Based on the effects of drought stress on the principal ecophysiological parameters, we were able to define three different levels of stress represented by thresholds in Ψ_{SWP} values. The first, Ψ_{SWP} above about -2.0 MPa, is characterized by the absence of stress. In this state, a wide variation in stomatal conductance is observed unrelated to Ψ_{SWP} variation.

Superimposition of environmental and plant or site specific factors affects stomatal behavior in well-irrigated trees.

Increased g_s in this state was not clearly improving leaf assimilation rates and, as a consequence, resulted in reduced iWUE.

Moderate stress was described by a Ψ_{SWP} between about -2 and -3.5 MPa, and was characterized by stomatal regulation of gas exchange. A reduction in water volume remaining in this range of potential improved iWUE, determining water savings. On the basis of the study conducted by Marra et al. [7], this stress level benefits oil quality without affecting system productivity. Finally,

below about -3.5 MPa, non-stomatal limits to photosynthesis are predominant. This represents a threshold value for high stress levels, which should be avoided because it is dangerous to the plant and has a marked and negative effect on both gas exchange and productivity.

Author Contributions: G.M. performed the experiments, analyzed data and wrote the paper. T.C. conceived and designed the experiments and revised the manuscript. L.F. edited the English and revised the manuscript. F.P.M. conceived and designed the experiment, analyzed data and revised the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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