agronomy

MDPI

Article

Root–to–Shoot Signaling and Leaf Water–Use Efficiency in Peach Trees under Localized Irrigation

Evangelos Xylogiannis [1,2], Adriano Sofo [1], Bartolomeo Dichio [1], Giuseppe Montanaro [1] and Alba N. Mininni [1,*]

[1] Dipartimento delle Culture Europee e del Mediterraneo, Università degli Studi della Basilicata, 85100 Potenza, Italy; evangelos.xylogiannis@zespri-europe.com (E.X.); adriano.sofo@unibas.it (A.S.); bartolomeo.dichio@unibas.it (B.D.); giuseppe.montanaro@unibas.it (G.M.)

[2] Zespri Fresh Produce Italy S.r.l., 04011 Aprilia (LT), Italy

* Correspondence: alba.mininni@unibas.it; Tel.: +39-0971-205-269

Received: 10 February 2020; Accepted: 18 March 2020; Published: 23 March 2020

Abstract: Global climate change is affecting important natural resources including water. Increasing temperature will change rate of evaporation and transpiration, leading to variations in water availability, ground water recharge, and water consumption by plants. Thus, competition for water will be a major future challenge for agriculture. Increasing water productivity at farm level is necessary to increase the efficiency of the irrigation system, plant water–use efficiency (WUE) and to optimize irrigation management. We test the hypothesis that in field–grown, drip–irrigated nectarine trees, the roots in the un–irrigated inter–row soil produce chemical signals that increase in summer to induce stomatal closure and so increase WUE. Concentrations of abscisic acid (ABA) were determined in leaf, root, and xylem sap of drip–irrigated (D) trees in which only about 25% of the soil volume was wetted and compared with those of trees irrigated using microjets (M) in which the whole soil volume was wetted. We also examined the effects of increased ABA on root–to–shoot dry matter ratio, the ratio ABA to indole–3–acetic acid (IAA), sap pH, and fruit and shoot growth. Both D and M trees were maintained at optimal water status as judged by pre–dawn leaf water potentials (about −0.3 MPa). There were no significant differences between treatments in mean fruit size (fruit diameter) or in tree yield (total fruit weight). However, shoot length was strongly reduced in D trees (to 75%) compared to M trees (100%). The concentrations of ABA in the inter–row roots of D trees were increased by 59% and that in the leaves by 13% compared to in the M trees. Despite the similar water status of D and M trees, a clear chemical signal was triggered in terms of a significant increase in the ABA/IAA ratio. This signal influenced leaf stomatal conductance which was 40% lower in D trees than in M trees. The associated responses in photosynthesis and transpiration raised the WUE of D trees by 7%–10% compared to M trees. This field study shows that in drip–irrigated trees, an ABA root–to–shoot signal issues from the inter–row roots growing in soil that dries out during a Mediterranean summer (hot, low rainfall). This ABA–induced WUE increase was achieved principally through reduced stomatal conductance and reduced transpiration.

Keywords: ABA; IAA; sustainable irrigation management; nectarine; hormonal interactions; drip–irrigation

1. Introduction

Abscisic acid (ABA) is among the main hormones participating in the root–to–shoot chemical signaling processes [1]. As a weak acid, ABA distribution among plant cell compartments is based on their pH levels, as elevated pH of sap in leaves promotes ABA accumulation in the apoplast and the guard cells. Under drought conditions, apoplastic pH increases, resulting in greater apoplastic

retention of ABA which then functions as a root−to−shoot signal leading to reduced transpiration in leaves [2,3]. When plants experience soil water depletion, chemical signals are generated that strongly affect the physiology, before any hydraulic signals are generated such as water potential changes [4]. Chemical signals appear early on with soil water shortage and can act prior to and independently of any hydraulic signals. Ultimately these chemical signals can increase plant water−use efficiency (WUE) [5,6].

ABA synthesized in the root is defined as a "long distance, non−hydraulic root signal" [7]. The effects of ABA as a drought−induced chemical signal can vary between species. For example, isohydric plants maintain stable leaf water potentials (Ψ) when well irrigated and also under drought conditions. Meanwhile, Ψ is more variable in anisohydric plants which maintain open stomata and high rates of photosynthesis under conditions of decreasing Ψ. In such plants there are cases where g_s is not affected by changes in ABA concentration [8–10].

Low soil moisture generally causes increases in ABA concentration in the root xylem. However, continued localized soil drying can limit ABA export to the shoot [11]. Increases in apoplastic pH result in greater accumulations of ABA in the apoplast, which ultimately closes stomata and decreases g_s [12]. Chemical signaling in plants can also be through changes in the balance between different hormones. The roles played by indole−3−acetic acid (IAA) as a chemical signal in plants are still unclear [13]. Thus, it is known that it can interact with ABA (expressed in the ABA/IAA ratio) to increase the synthesis of ABA [14]. IAA was the first substance identified as an auxin and it is the most abundant form of this hormone in several plants [15]. It was also confirmed that the phytohormones indole−3−acetic acid (IAA), abscisic acid (ABA), and several cytokinins have important functions in developmental stages and growth of *Prunus* spp. [16].

During the last few decades, a large number of irrigation methods and irrigation management techniques have been proposed to supply water at a level somewhat below the plant's full water requirements. These can increase plant productivity per unit of water [17] and also plant WUE by regulating various physiological processes (e.g., leaf gas exchange) through the generation of root−to−shoot signals [18–20]. Among the methods aimed at increasing water conservation, regulated deficit irrigation (RDI) [17,21,22] and partial root−zone drying (PRD) are the most popular [6,23–26].

Localized irrigation (e.g., drippers) can supply full or even excess water that is enough to keep the trees at optimal water status as assessed through leaf/stem water potential [22]. In drip−irrigated orchards, the Mediterranean dry season inevitably generates gradients of soil moisture between the row (irrigated, wet) and the inter−row (un−irrigated, dry) soil to the extent that the inter−row soil moisture can fall close to the wilting point [27]. Fernández and colleagues [28] showed that such a high spatial variability in soil moisture may impair some water relation parameters in olive. They hypothesized a chemical signal sourced from the roots in the dry zone. In line with this idea, an ABA−induced signal originating in the roots in the dry zones of the soil has been detected in potato [29]. The possible effect on chemical signaling of soil moisture variation in the inter−row soil has not been well explored in tree crops. Working with nectarine trees that are fully drip−irrigated within the row but un−irrigated in the inter−row, this study tests the hypothesis that the roots in the inter−row will produce a chemical signal during the dry summer months that induces stomatal closure and, hence, a decrease in g_s and an increase in WUE. To test this, we measured ABA and IAA along with key water relations parameters in well−irrigated trees under either drippers (localized) or microjets (wetting the whole soil surface) during a typically dry Mediterranean summer. We also examined the effects of the putative chemical signal on the growth of the fruit and shoot, and on the ratio of leaf to root.

2. Materials and Methods

2.1. Experimental Design and Plant Material

The study took place during the 2012 and 2013 growing season at a nectarine orchard (*Prunus persica* L., cultivar Big Bang grafted on GF677) located in Metaponto, south Italy (40° 23'58,

13″ N; 16° 45′43, 50″ E). Trees were planted in 2008 in rows every 1.5 m with a 4.0 m inter−row (1666 trees ha^{-1}) and trained to delayed−vase. The area has a typical Mediterranean climate with dry summers and average annual rainfall of 550 mm (SAL Service, ALSIA Basilicata Region). The orchard was managed according to local commercial practices. Average soil pH was 7.3, the texture of the soil (0–20 cm depth) was 70.6% sand, 16.2% loam, and 13.1% clay. Field water capacity (FWC) and permanent wilting point (PWP) (0–60 cm soil depth) were 15.10% and 5.95% (v/v), respectively. The difference between FWC and PWP was used to calculate the available water (AW).

During the first year of the experiment, two blocks of 48 trees each were identified (four rows) and used for comparisons between two the irrigation methods. From the central rows of each block, seven trees were selected for eco−physiological parameters and 10 trees for yield determination. The two blocks were irrigated either by drippers (D) or by microjets (M). The D irrigated trees were supplied by two drippers per tree each rated at 8 L h^{-1}, so delivering 16 L h^{-1} $tree^{-1}$, but wetting only about a 1 m wide volume of soil along the row. This narrow in−row strip of soil represented only about 25% of the soil area within the rootzone of the trees—it is estimated about 75% of the rootzone was rainfed only. Meanwhile, M irrigated trees were supplied by emitters (35 L h^{-1} $tree^{-1}$), ensuring the wetting of the whole soil surface above the rootzone—i.e., the row plus the full inter−row. See Figure S1 of the Supplementary Material for a schematic view of the irrigation treatments and soil wetted and excavated area and Figure S3 for irrigation volumes applied.

The irrigation needs were calculated based on reference evapotranspiration (ET_0) and adjusted for the rare rainfall events. Source meteorological data were obtained from a weather station about 2 km from the experimental orchard. The calculation also employed a crop coefficient (K_c) previously developed for this area [22]. Irrigation frequency was adjusted so as to keep the soil moisture in the top 50 cm soil layer within range of 50% of AW, determined based on the soil properties (see above). During the first year, the set−up of the experiment and preliminary analysis were performed, while all measures were carried out during the second year. This paper reports results related to the second year (2013).

2.2. Soil Moisture

Soil moisture was monitored throughout the experimental period by soil samples (0–20 cm depth) taken within the row and within the inter−row every 15–20 days from two trees per treatment. Soil samples were first weighed to obtain the fresh weight (FW) and then dried to constant dry weight (DW) at 110 °C for 48 h in a ventilated oven. Water content (% w/w) was calculated as 100 × (FW - DW)/DW.

2.3. Leaf to Root Ratio

Whole leaves were collected at leaf fall (end of September) by placing nets on the ground beneath six trees per treatment. Leaf DW was determined by drying to constant weight in a ventilated oven at 110 °C for 48 h.

Roots were excavated from three trees per treatment, chosen from among those used for the leaf dry weight determination. A backhoe was used to excavate trenches 2.0 m from each tree to a depth of about 0.9 m. A single block of soil (approx. 30 × 30 × 30 cm) was then removed from within a 2 × 1.5 m area around each tree (this area represents half of the total roots for a tree, Figure S1). The soil samples were sieved by hand and roots were collected carefully, washed gently with distilled water, wrapped in paper, and promptly transferred to the laboratory.

After determining root sample FW, roots were dried to constant weight in a ventilated oven to determine root DW. Total root DW per tree (to a depth of 30 cm) was then estimated by dividing the DW of the excavated (30 × 30 × 30 cm) root sample by the ratio (0.015) of the sample area (30 × 30 cm = 0.09 m^2) to that of the whole tree (2 × 1.5 × 2 m = 6 m^2). The leaf to root dry matter ratio was then calculated as kg kg^{-1}.

2.4. Shoot and Fruit Growth, Yield, and Fruit Quality

Shoot growth was measured weekly during the growing season (from May to August) on 60 selected, elongating (i.e., current year) shoots on 15 branches chosen randomly on six trees per treatment.

Fruit diameters were measured with digital calipers weekly during the growing season on the same branches used for shoot−elongation measurement. About 50 fruits were labelled and measured per treatment.

Harvest was on day of year (DOY) 159 (June 8). Fruit yield FW was estimated on 10 trees per treatment by picking and weighing all fruits. Sub−samples were promptly transported to the laboratory for the determination of various fruit−quality traits.

Fruit firmness at harvest was measured with a penetrometer (Nicesound UFL−031; Nicesound Electronics Co., Zhejiang, China), using an 8 mm diameter probe. Soluble sugar content (expressed in °Brix) was determined for each of 50 fruits per treatment using the expressed juice and a refractometer (NWL−32, Novel Ways Ltd., Hamilton, New Zealand).

2.5. Leaf Gas Exchange, Water−Use Efficiency, and Water Potential

Leaf gas exchange was measured on DOY 204 (July 23) and 220 (August 8) using an open−flow portable system (LI−6400; Li−Cor Inc., Lincoln, NE, USA) equipped with a leaf chamber fluorometer (LI−6400−40; Li−Cor Inc., Lincoln, NE, USA) operating at 500 μmol s^{-1} flow rate. The measurements of gas exchanges were carried out from 06:00 to 18:00 h every 2–3 h on 15 fully expanded, horizontally positioned leaves (five leaves per tree) on three trees per treatment. The temperature in the leaf chamber was maintained equal to the external air temperature by the instrument's automatic temperature adjustment. Leaf WUE was calculated as units of assimilated CO_2 per unit of transpired water by dividing net photosynthesis (A) (μmol CO_2 m^{-2} s^{-1}) by transpiration (E) (mmol m^{-2} s^{-1}). The WUE values calculated at various times of day were combined to give an average daily value.

Stem water potentials were measured pre−dawn (Ψ_{pd}) and at midday (12:45–13:30 h) (Ψ_s) on 18 leaves per treatment sampled from six trees per treatment using a Scholander pressure chamber (Model 600; PMS Instrument, Albany, OR, USA). Before the Ψ measurements, leaves had been covered with aluminum foil for at least 1 h to minimize transpiration. During DOY 204 and 220 the diurnal course of Ψ_S was determined from measurements made every 2–3 h from 06.00 to 18.00 h.

2.6. Xylem Sap Sampling and pH Determination

On DOY 172 (21 June), 185 (4 July), and 220 (8 August), xylem sap was collected from three, one−year−old shoots per tree (three trees per treatment). Shoots were collected pre−dawn and promptly defoliated and debarked to avoid phloem sap contamination. A silicon rubber tube was connected to the cut end of the shoot and the shoot sealed in a pressure chamber with the tube passing through the rubber septum to the outside to allow collection of the xylem sap. The applied pressure was increased gradually at around 0.1 MPa min^{-1} eventually reaching a maximum of 3 MPa. To avoid possible contamination from cut cells, the first drops of exuded xylem sap were rejected. Subsequent volumes of sap were collected in a small receptacle covered with aluminum foil to minimize evaporation and cooled by ice to slow any degradation.

Xylem sap samples of 200–300 μL were collected during the first 10 min under pressure and immediately stored at −80 °C. Later, the pH values of the xylem sap samples were measured (pH meter, SevenGo due tmpro−sg78−b pH meter/cond; Mettler−Toledo S.p.A, Milan, Italy).

2.7. Hormone Extraction and Determination

During the first year of the experiment (2012) the methods for hormonal extraction were adapted from Sofo et al. [16], while hormonal analysis was tested according to Lovelli et al. [30]. During the second year (2013) hormonal determinations were carried out on samples collected on the same days

as the soil and xylem sap samplings i.e., at 8:00 h on DOY 172, 185, 220. Roots from the row and inter−row positions were collected for each irrigation treatment (to 20−cm depth) from three plants per treatment. Roots of diameters 1.5–3.0 mm were gently washed free of soil, wrapped in aluminum foil, and promptly stored at −80 °C in an insulated (expanded polystyrene) box. At midday, three leaves from a height of about 1.80 m and well exposed to the sun were collected from three trees per treatment. The same storage procedure was used as for the root samples.

For extractions of ABA and IAA, a 250 mg sample of shoot or root tissue was ground to a powder with liquid nitrogen using mortar and pestle and placed in a tube to which 2.5 mL of extraction solvent (2−propanol/H2O/HCl 37%; 2:1:0.002, v/v/v) was added. The tubes were shaken at 100 cycles per minute for 30 min at 4 °C (Thermo−shaker Biosan, TS100C). To each tube, 2.5 mL of dichloromethane was added, and the samples were then shaken as above for a further 30 min at 4 °C and centrifuged at 20,800× *g* for 5 min (refrigerated centrifuge Eppendorf 5427R). After centrifugation, two phases separated out, with plant debris lying between the two layers. Next, 1.0 mL of the solvent from the lower phase was transferred to a screw−cap vial using a Pasteur pipette and the solvent mixture was concentrated using an evaporator with nitrogen flow. Finally, the samples were re−dissolved in 0.1 mL methanol and stored at −20 °C pending quantitative analysis. The quantitative determination of ABA and IAA concentrations in the roots, leaves, and xylem sap was carried out by a competitive enzyme linked immunosorbent assay (ELISA) using the Phytodetek®ABA Test Kit (Agdia Biofords, Evry, France). The means of the optical densities and binding percentage of duplicate standards or samples (100 L) were calculated and plotted in a semi−logarithmical scale, in order to linearize the equation [16].

2.8. Statistical Analyses

All experimental results were analyzed statistically using Minitab 16 (Minitab Inc., State College, Pennsylvania). Comparisons between treatments (D, MJ) per day were carried out using the two sample *t*−test for equal means [31] for physiological measures, p−values < 0.05 were considered significant. Hormonal results were analyzed by two−way ANOVA considering treatment/row and inter−row as first factor and sampling time as second factor. The number of real statistical replicates (n) is indicated in the caption of each table and figure, all data are reported as means and standard errors of the mean (± SE).

3. Results

3.1. Environmental Conditions and Soil Moisture

During the experimental period, daily ET_0 followed a typical Mediterranean pattern, ranging from 4.5 (May) to 7.5 mm d^{-1} (August) with some peaks of approx. 8 mm d^{-1} recorded on DOY 173 (22 June), 209 (28 July), and 217 (5 August) (Figure S2). The VPD followed a similar pattern ranging from a minimum of about 0.5 to a maximum of about 3 kPa. Rainfall occurred on a number of days during the period, with maxima on DOY 178 (27 June) and 193 (12 July) (8.2 mm on each day). The total rainfall over the experimental period was 38.6 mm, with the last rainfall occurring on DOY 195 (14 July) (Figure S2).

At the beginning of the growing season, soil moisture (0–20 cm depth) was at FWC in both the row and inter−row areas of both the D and M blocks. Throughout the experimental period, the soil moisture levels of the row soil of both blocks was maintained roughly constant at FWC (data not shown). As expected, in the inter−row the different irrigation protocols resulted in widely different soil moisture levels between the D and M blocks. Soil in the D inter−row was not irrigated, and soil moisture levels declined from the end of April, reaching a minimum of about 40% of FWC at the beginning of June. After this it declined gradually to about 30% of FWC at the beginning of July (Figure 1). As the inter−row soil of the M block was irrigated, soil moisture here ranged between about 80% and 95% of FWC throughout the experimental period (Figure 1).

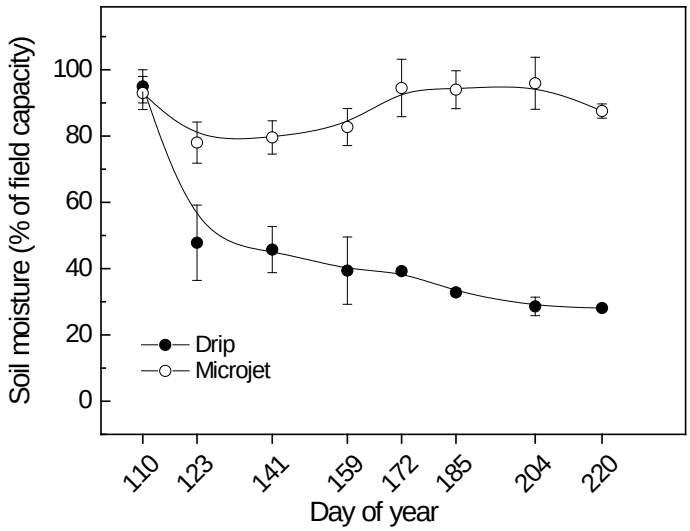

Figure 1. Average soil moisture (% of field capacity) measured at the inter−row position in drip (•) and microjet (○) blocks at 0–20 cm soil depth (n = 3) during the experiment. Lines are illustrative only.

3.2. Water Status, Vegetative and Productive Traits

Measured values of Ψ_{PD} were similar in the D and M trees on most days, being on average about −0.3 MPa (Figure 2). Values of Ψ_S followed a similar pattern in the D and M trees, being on average about −1.1 MPa on DOY 149, then recovering somewhat to reach significantly different values at the end of June of −0.88 (D trees) and −0.67 MPa (M trees). Thereafter, Ψ_S gradually decreased until the end of the experiment when significantly different values were again detected on DOY 204 (23 July), being −1.4 (D trees) and −1.16 MPa (M trees) (Figure 2).

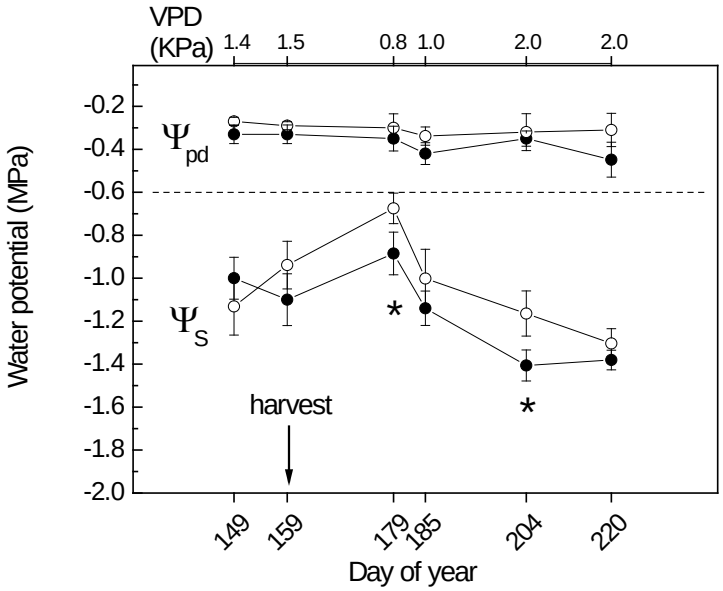

Figure 2. Pre−dawn (Ψ_{PD}) and midday stem (Ψ_s) water potential (n = 18) measured in some days during the experimental period in drip (•) and microjet (○) irrigated trees. Values of vapor pressure deficit (VPD) were recorded at midday; Harvest day, June 8th; * indicates statistically significant difference between treatments at $p < 0.05$.

Fruit size (diameter) was not significantly different between the two treatments at any stage during growth with comparable values on each day of measurement (Figure 3A). In contrast, shoot

length was significantly affected by the irrigation treatments being longer in the M trees than in the D trees throughout the season (Figure 3B).

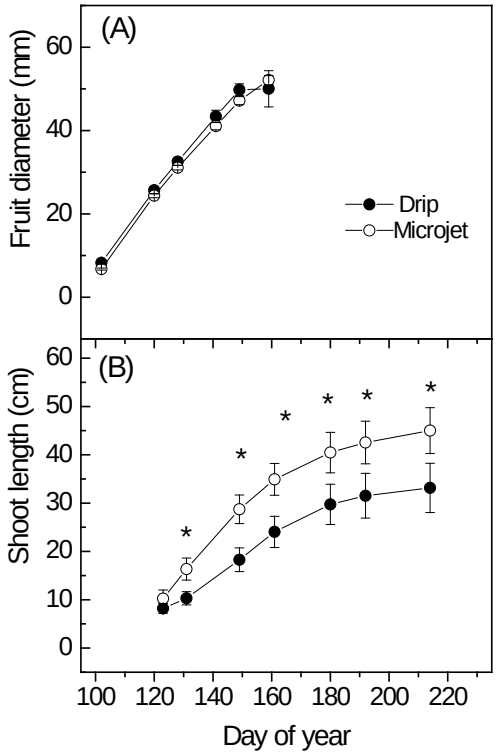

Figure 3. Development of (**A**) fruit diameter (n = 50) and (**B**) shoot length (n = 60) measured in trees under drip (•) and microjet (○) trees. Comparing treatments within the same parameter, * indicates statistically significant differences.

At harvest (DOY 159, June 8), fruit yield and individual fruit fresh weight of the D and M trees were similarly at about 13 kg tree^{-1} and 130 g fruit^{-1}, respectively (Table 1). However, fruits collected from the M trees were significantly firmer and with lower soluble sugar contents than those from the D trees (Table 1).

Table 1. Mean valued (± SE) of the irrigation volume (m^{3}tree^{-1}) and yield (fresh weight—FW) per tree (n = 6), fruit fresh weight (FW) (n = 50), fruit firmness and soluble sugar (n = 50) in fruits collected on 8 June, 2013 from drip and micro−jet irrigated trees. Comparing treatments within each parameter different letters indicate a statistically significant difference at $p < 0.05$.

Irrigation Method	Irrigation Volume (m^3/tree^{-1})	Yield (kg FW Tree^{-1})	Fruit FW (g Fruit^{-1})	Firmness (kg cm^{-2})	Soluble Sugar (°Br)
Microjet	9.19	13.92 ± 2.36a	132 ± 0.0031 a	3.84 ± 0.15 a	9.52 ± 0.17 a
Drip	3.14	13.16 ± 1.09a	134 ± 0.0027 a	3.43 ± 0.13 b	9.98 ± 0.19 b

Total leaf dry matter was markedly different in the M and D trees being about 2.4 and 0.57 kg tree^{-1}, respectively. The estimated total root dry matter was 1.54 and 1.49 kg tree^{-1} for D and M trees, respectively. These values generate a leaf−to−root ratio four times higher in the M trees than the D trees (Figure 4).

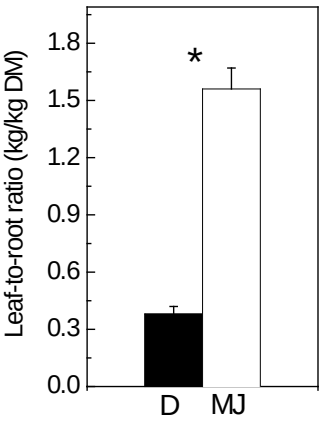

Figure 4. Leaf−to−root ratio measured on dry matter (DM) basis in trees (for leaves, n = 6; for roots n = 3) under drip (D) and microjet (MJ) irrigation, * indicates statistically significant differences.

3.3. Leaf Gas Exchange

Leaf gas exchange showed a typical daily pattern. On DOY 204 (July 23), A, E, and g_s tended to be similar between the treatments during the early part of each day but from midday onwards values were significantly higher in the D trees, compared to the M ones (Figure 5C).

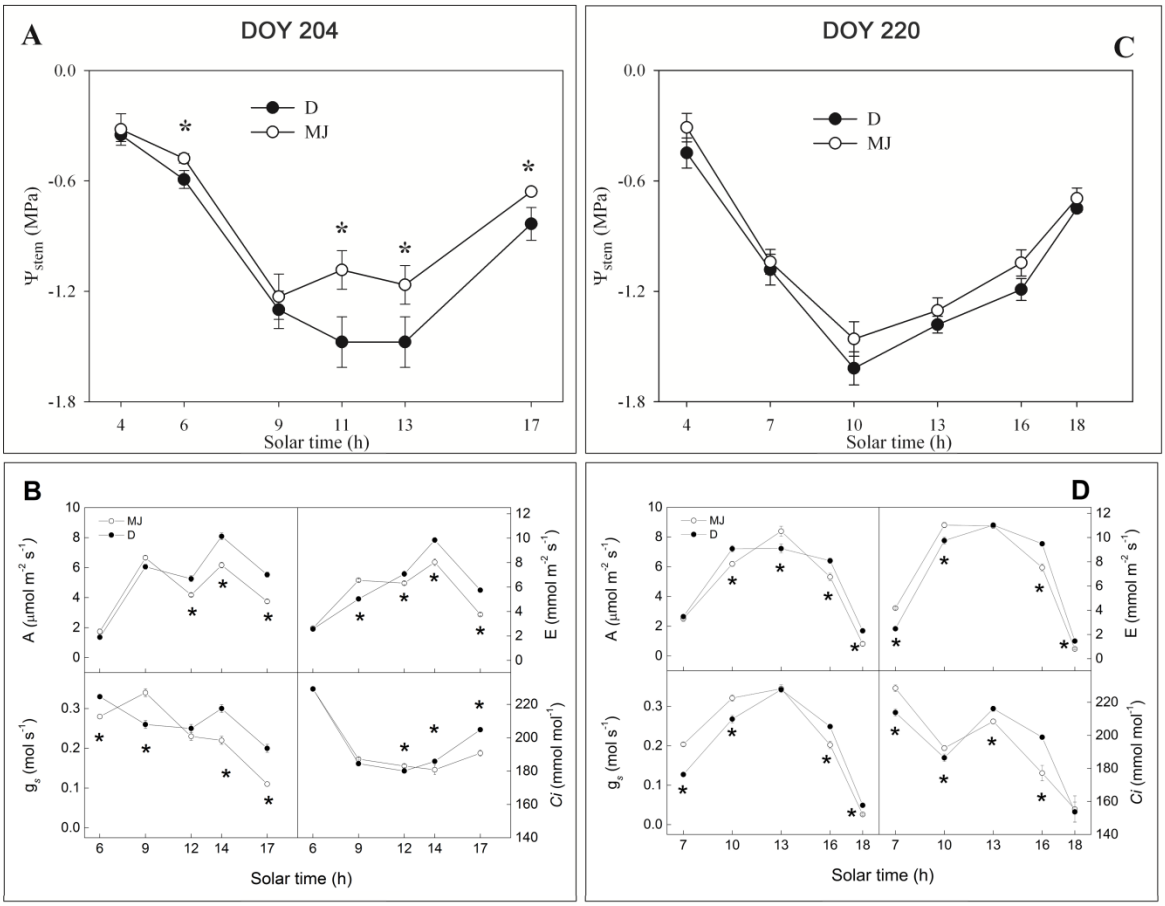

Figure 5. Stem water potential (Ψ_s) and leaf gas exchange (net photosynthesis = A, leaf transpiration = E, stomatal conductance = g_s, and intercellular CO_2 concentration = C_i) (n = 15) on day of year (DOY) 204 (23rd July) (**A**,**C**) and DOY 220 (8th August) (**B**,**D**) in drip (•) and micro−jet (○) irrigated trees, * indicates a statistically significant difference between treatments at $p < 0.05$. For some points, error bars are smaller than the symbol size.

On DOY 220 (August 8), morning values (7.00–10.00 h) of the gas exchange parameters tended to be significantly lower in the D trees than in the M ones but later in the day, values of A, E, and g_s were higher in the D trees (Figure 5D).

On average, the daily leaf WUE values were significantly higher in the D trees than in the M ones (Figure 6).

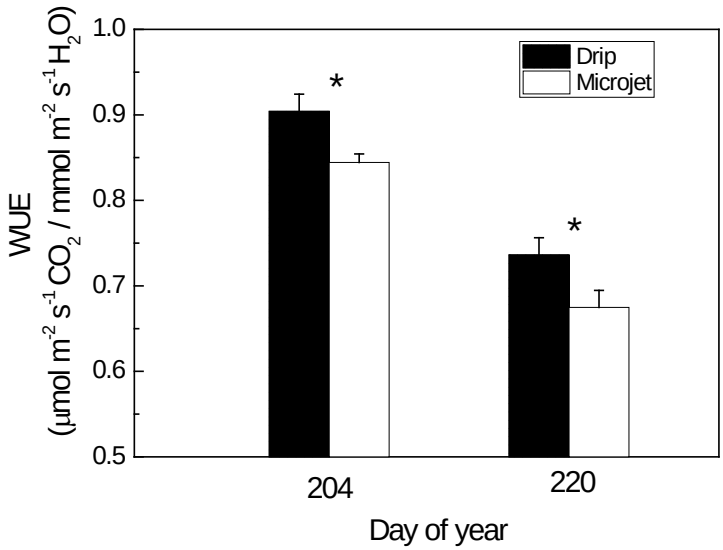

Figure 6. Leaf water−use efficiency (WUE) measured in leaf of drip and microjet irrigated trees at DOY 204 (23rd July) and DOY 220 (8th August). * indicates a statistically significant difference (n = 15).

3.4. ABA

Root ABA concentration was at similar values between the two treatments and in the two soil positions on the first day of measurement on DOY 172 (21 June) but after DOY 185 (4 July) it increased approximately 3.6−fold in the M inter−row (Figure 7A). Root ABA concentration of the D inter−row increased after the first sampling approximately 5−fold (10.84 pmol g^{-1}). On average it was about 1.5−fold higher than in the D row and about 3− and 2−fold higher than in the roots sampled from the M row and M inter−row, respectively (Figure 7A). In the D row, root ABA concentration peaked on DOY 185, being significantly higher than the root ABA concentrations in the two other samplings. For all sampling days (but in particular for the last two) the values of ABA concentration in the D inter−row roots were about 2−fold higher than in the M inter−row roots (Figure 7A).

The level of ABA measured in the xylem sap was not significantly different between the irrigation treatments at any of the three sampling days. Additionally, xylem sap ABA concentration was similar at about 100 pmol mL^{-1} in the first two sampling days (DOY 172 and 185), whereas on DOY 220 it decreased by 98.6% (to about 1.5 pmol mL^{-1}) and 91% (about 2.8 pmol mL^{-1}) in M and D plants, respectively (Figure 7B).

Leaf ABA concentration was significantly higher in the D trees than the M trees at all the three samplings. In both treatments, leaf ABA concentration on DOY 185 was lower than that measured on the previous sampling day. Leaf ABA concentration reached its highest values of about 41 (D) and 33 (M) pmol g DW^{-1} on DOY 220 (Figure 7C).

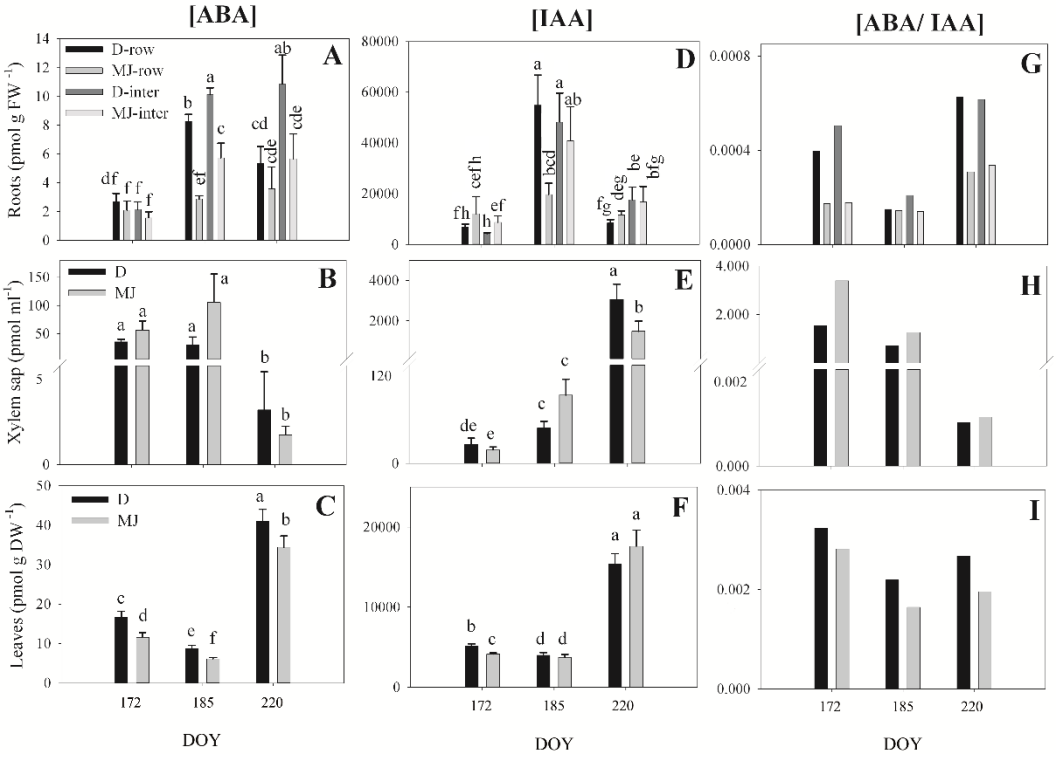

Figure 7. (**A**) Abscisic acid [ABA], (**D**) indole−3−acetic acid [IAA], and (**G**) abscisic acid/indole−3−acetic acid concentration ratio [ABA/IAA] in roots of the row (black bars) and inter−row (light black bars) positions of the drip−irrigated block, and in roots of the row (gray bars) and inter−row (light gray bars) positions of the micro−jet irrigated block. (**B**) Xylem sap (ABA), (**E**) xylem sap (IAA), (**H**) xylem sap (ABA/IAA), (**C**) leaf (ABA), (**F**) leaf (IAA), and (**I**) leaf (ABA/IAA) in drip (black bars) and micro−jet (gray bars) irrigated trees. Different letters indicate statistically a significant difference at $p < 0.05$ ($n = 3$). Error bars represent SEM.

3.5. IAA

The concentration of root IAA was consistently low in the M row soil (Figure 7D) but increased about 4.7−fold in the M inter−row by DOY 185 and by about 8.0− and 11.3−fold in the D row and D inter−row roots, respectively (Figure 7D). On DOY 220, IAA concentration decreased to reach similar levels to those measured on DOY 172 in the row soil of both D and M treatments. However, IAA concentrations were higher for roots sampled from the inter−row soil of both D and M treatments (Figure 7D). For both D and M treatments, xylem sap IAA concentration increased during the season, showing a notable increase by DOY 185 of 98.6% and 94.3% for D and M trees, respectively, being 51.6% significantly higher for D trees (Figure 7E). In leaves, IAA concentrations showed similar trends to the xylem sap, with similar values during the first two sampling but increasing by DOY 220 to approximately 74.1% and 78.8% for the D and M trees, respectively. A statistically significant difference occurred only on the first sampling day when the leaves of the D trees had higher IAA concentrations than the M trees (Figure 7F).

3.6. ABA/IAA Ratios and Xylem Sap pH

On DOY 172, compared to the M row and M inter−row, the ABA/IAA ratio was approximately 55.6% and 64.6% higher in the D row and D inter−row roots, respectively (Figure 7G). On DOY 185 the root ABA/IAA ratio decreased in the D row, to reach similar values to the values in the M row and M inter−row. However, in the D inter−row the root ABA/IAA ratio remained 28.4% higher. On the last sampling day, the ABA/IAA ratio in the roots reached its highest values, and roots of the D trees showed about 48% higher values than of the M trees (Figure 7G). The root xylem sap ABA/IAA ratio

decreased throughout the experimental period, showing consistently lower values in the D trees than in the M ones (Figure 7H). In leaves, the ABA/IAA ratio was higher for the D trees at all three days of sampling and fell to its lowest values on DOY 185 in both treatments (Figure 7I).

In the D trees values of xylem sap pH remained lower than in the M trees throughout the sampling days, with significantly lower values on DOY 185 (pH 5.73 and 5.95 for D and M trees, respectively) and on DOY 220 (pH 5.62 and 5.80 for D and M trees, respectively) (Figure 8).

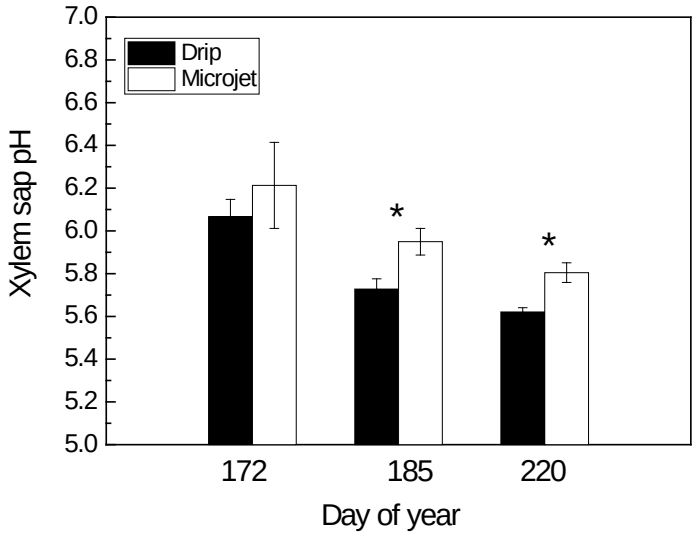

Figure 8. Xylem sap pH (n = 9) in drip and micro−jet irrigated trees. * indicates a statistically significant difference ($p < 0.05$) between treatments at the same day of year.

4. Discussion

4.1. Tree Water Status, Shoot and Fruit Growth, and Root ABA Concentrations

Although drip irrigation maintained the D trees at a near optimal water status, their leaf ABA concentrations were increased compared to the M ones. This is because of increased ABA concentrations in the roots exploring the un−irrigated soil in the inter−row. Interestingly, tree water status, as measured through Ψ_{PD}, was substantially similar in trees of both the D and M trees and was roughly stable throughout the season (Figure 2). Meanwhile, the values of Ψ_S in both treatments varied according to variations of VPD (Figure 2).

The assessment of midday tree water status reveals a tendency for M trees to express less negative values of Ψ_S than D trees on just a few occasions. This suggests that Ψ_S may be a more reliable measure of overall tree water status than Ψ_{PD} [30–33]. However, because the maximum differences in Ψ_S between the D and M trees were always quite small (about 0.15 MPa) and appeared only somewhat erratically, and also because their Ψ_{PD} values were always substantially the same, it seems reasonable to infer that most of the time the D trees, like the M trees enjoyed near−optimal water status [34].

Due to the known sensitivity of fruit growth to sub−optimal tree water status [35], our findings of similar fruit development and yield in D and M trees (Table 1, Figure 3A) further confirms that both sets of trees enjoyed similar and near optimal water status. Maybe the transiently lower Ψ_S values in the D trees (Figure 5) can be interpreted merely as a temporary inefficiency of the hydraulic system to supply the canopy. Although tree water status and fruit growth measurements suggest close to optimal conditions of irrigation, shoot elongation was significantly lower in the D trees than in the M ones, with final shoot lengths being shorter by 27% (Figure 3). Recognizing that shoot elongation is suppressed by ABA [7], our significantly slowed shoot growth, although tree water status was similar, suggests a non−hydraulic regulation in the D trees—i.e., the suppression could be ABA−induced. This fits with the increased ABA concentrations found in our leaves during the final stages of shoot elongation (Figures 3 and 7C).

The synthesis of ABA in water−stressed roots can cause ethylene inhibition, and lead to increased root growth [36]. It can also reduce leaf area growth as the hormone is transported to the above−ground parts of the tree [37,38]. We found the increased ABA was associated with a strong reduction in leaf−to−root ratio in the D trees—to only about 25% of that in the M trees (Figure 4). This response has been noted in a number of studies—especially for plants growing in drying soils [38,39]. The lower leaf−to−root ratio may also help explain the significantly higher ABA concentrations in the leaves of D trees, despite the similar ABA concentrations measured in their xylem saps (Figure 7B,C). Chemical signaling can affect tree growth and stomatal behavior, even when water status is unaffected [40]. These effects can help trees adapt to drying soils, as lowered leaf area reduces transpiration and also uses up less assimilate. This in turn leads to an increase in root biomass and also in the volume of soil explored by the roots [41].

In the D trees, the ABA concentration varied between the row and inter−row roots (Figure 7A) in line with the soil moisture variation (Figure 1). The root ABA concentration was increased in roots exploring the drying soil of the inter−row in the D trees on two sampling dates (DOY 185 and 220) (Figure 7A). However, on DOY 185 in the D trees, the ABA concentration also increased in the roots in the (wet) volume of soil within the row. The xylem sap from these peripheral, inter−row, roots must pass through the roots lying in the row as it moves toward the leaves.

Our results confirm that under localized (dripper) irrigation, the inter−row roots are exposed to drying soil conditions and produce a chemical signal (increased ABA) that affects shoot growth and leaf gas exchange. This suggest physiological studies of drip−irrigated trees should include some consideration of chemical signaling from the un−irrigated inter−row roots.

4.2. Gas Exchange, Xylem Sap pH, and Hormones

The diurnal variations in leaf gas exchange reported here are in line with previous observations in the same area on drip−irrigated peach trees [22,42]. Our results reveal different gas exchange behaviors between our D and M trees, depending on the time of day. The D trees generally showed lower values of A, E, and g_s before midday, except the value of A at 10 am on DOY220 and g_s at 6 am on DOY 204, and higher values during the afternoon (Figure 5C,D).

The ABA concentrations in the roots of the D trees exploring the un−irrigated soil were double those in the row (Figure 7D). This significantly increased the leaf ABA concentrations compared with the M trees (Figure 7C). As expected, even though the Ψ_s values were similar in the two treatments, the increased ABA in the D trees reduced the values of g_s and E, especially during the morning period (Figure 5). We did not record possible daily oscillations in ABA concentration [8,43]. Nevertheless, our failure to detect ABA−induced stomatal closure in our D trees in the afternoon may have been due to a reduction in ABA concentration (Figure 5C,D). Further work should be directed to ascertain the daily pattern of ABA metabolism/catabolism in drip−irrigated trees.

The role of dry roots in drip−irrigated but well−watered trees as a source of some sort of chemical signaling was hypothesized by [28] to explain the approx. 30% reduction in daily transpiration recorded in locally−wetted olive trees (dripper irrigated) compared to ones where the whole rootzone was wetted (pond−irrigated). Our results support and to some extent explain this observation while also showing there is little or no negative effect on fruit yield or quality but a significant saving in irrigation water usage.

Zhang and Davies (1990) [44] indicated that increases in xylem sap [ABA] can occur before its increase is noticed in the leaves, as observed in our experiment (Figure 7B,C). Furthermore, xylem ABA transport is slow and based on a specific carriers, and it can vary among a huge range of concentrations, having small or even negligible effects on g_s [43]. Leaf [ABA] increased in the last sampling date DOY 220 (8 August) (Figure 7C), demonstrating the slow kinetic of the chemical root−to−shoot signal compared with the movement of electrical signals or Ca^{2+} waves, and following an increase in ET_0 and VPD (Figure S2) [45].

ABA is a weak acid hormone and it could be transported from relatively low pH to high pH conditions without specific transporters, via a passive diffusion mechanism (i.e., by pH gradients) [41]. For this reason, its accumulation and movement across different plant compartments (e.g., apoplast, guard cell, stomata, cytosol) depends on their pH levels, as elevated pH of sap in leaves promotes ABA accumulation in the apoplast and the guard cells [46,47]. In this work, a decrease of the pH of the xylem sap collected from shoots was noticed in the leaves of trees from both the treatments, with values in D trees significantly lower on DOY 185 and 220 (Figure 8). This reduction of xylem sap pH was parallel to decreases in xylem sap [ABA] (Figure 7B) and increases in leaf [ABA] on DOY 220 (Figure 7C). It could be hypothesized that the lower values of xylem sap pH in shoots, promoted the xylem ABA transport toward the leaves (Figure 7B,C and Figure 8). This is in accordance with previous works [48,49], who observed that high pH in shoot xylem sap enhanced the accumulation of ABA in roots as a result of its lower transport to other departments (e.g., leaves). Consequently, xylem sap [ABA] had an opposite trend of leaf [ABA] in all the sampling dates (Figure 7B,C). The relationship between sap pH and leaf [ABA] reveals that shoot xylem sap can function as a sink of ABA, that finally reaches leaves and guard cells, where it contributes to regulate stomatal function and g_s (Figure 5C,D).

Unlike ABA, IAA is mainly synthesized in the shoot tips, leaves, and flower buds, and its transport is primarily basipetal (from leaves to roots). In this work, root [IAA] increased by DOY 185, when roots from the row of the D trees had significantly higher values compared to those of the M−row (Figure 7D).

The [IAA] peaks for roots, xylem sap, and leaves were different in the various sampling dates. Indeed, on DOY 172 tissues showed low [IAA], whereas on DOY 185 when roots were likely at their maximum growth rates [50], root [IAA] was high and xylem/foliar [IAA] low, and finally on DOY 220, when leaf area reached its maximum expansion [51], [IAA] was high in both xylem sap and leaves (Figure 7E,F). On DOY 185, when no fruits were on the trees, a high root [IAA] was detected, particularly in D trees (Figure 7D). Noteworthy, this increase also followed a 15 mm rainfall (Supplementary Material, Figure S2) that occurred five days before that sampling date and probably contributed to root growth and [IAA] accumulation. Moreover, this increase in [IAA] could have been facilitated by the high [ABA] in the root tissues found in the same day, as ABA accumulation can modulate IAA transport in the root tip [36].

On DOY 185, [IAA] of both xylem sap and leaves was substantially lower (Figure 7E,F), likely indicating the higher transport of this hormone to the roots. On DOY 220, root [IAA] decreased (Figure 7D), while an increase of this hormone was noticed in both xylem sap and leaves (Figure 7E,F). This led to a kind of balance between the concentration in roots (≈13,600 pmol g^{-1} FW) and that in leaves (≈10,000 pmol g^{-1} FW). In roots and leaves, [ABA/IAA] ratio was higher in D trees, especially on DOY 172 and 220, indicating even more the role of ABA in the root−to−shoot signaling (Figure 7G,I). In xylem sap, this ratio was lower in D trees compared to the M ones (Figure 7G). This correlation between the two hormones could have affected the leaf−to−root ratio (Figure 4). The even small differences in firmness and soluble sugar content among fruits from the two treatments could be due to a slightly shifted ripening time induced by the higher vigor as suggested by the higher leaf biomass of the M trees (Table 1, Figure 4).

Drip−irrigated trees are usually considered as non−stressed trees based on their optimal Ψ [17,20]. However, our results suggest that in drip−irrigated nectarine trees, despite a roughly optimal Ψ_{PD} and Ψ_S (Figure 2) the increase in [ABA] (Figure 7A–C), and consequently in ABA/IAA ratio (Figure 7G–I), in not−irrigated roots (D−inter) allowed drip−irrigated trees to achieve a better compromise between stomatal closure and water loss by transpiration (Figure 5B,D). This behavior leads to an increased WUE (Figure 6) and, thus, to a reduction of irrigation requirements and a consequential water saving.

5. Conclusions

Our results suggest that when irrigation is very localized, particularly in our semi−arid growing environment, a root−to−shoot chemical signal arises in the unirrigated roots of the inter−row. This

signal influences stomatal conductance, so reducing transpiration and shoot growth and leaf−area growth. Our results on ABA−induced leaf WUE increases in the D trees support the conclusion that under our semi−arid conditions, wider usage of localized irrigation will increase whole canopy WUE and reduce the water requirement but with no loss of either fruit yield or fruit quality. This knowledge will benefit irrigated horticulture in many areas of the world where the availability and cost of irrigation water limits productivity by allowing either or both the saving of precious water or the moderate expansion of the industry for the same water usage.

Our results also indicate the potential for new research, in particular for work to elucidate the physiology associated with diurnal patterns of change in ABA biosynthesis and shoot response.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/3/437/s1, Figure S1: Schematic representation of the drip and microjet irrigation treatments and of wetted and excavated soil area, Figure S2 Daily values of rainfall (black bars) and daily reference evapotranspiration (ET0) (—) and vapor pressure deficit (VPD) (—) from DOY 141 to 220 (20th May to 8th August 2013), Figure S3: Irrigation volumes per tree during the experimental period (20 May–8 August 2013) for D (black column) and MJ (grey column) irrigated blocks.

Author Contributions: Conceptualization and supervision, B.D.; methodology, formal analysis, and data curation G.M., A.S., E.X., and A.N.M.; writing—original draft preparation, G.M., A.S., E.X., and A.N.M.; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This study was prepared within the Basilicata Region PSR 2007−2013 Programme (Misura 124, OTIROL, n. 6/2012) and with the contribution of the LIFE+ financial instruments of the European Union to project LIFE14 CCA/GR/000389 LIFE AgroClimaWater. We thank A. Mossuto, A. Tuzio, I. Arous, V. Valentini, G. Lillo and. J. Duràn for field support. G.M. was financially supported by an RTDb research contract (n. 06/2016).

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

References

1. Wilkinson, S.; Davies, W.J. ABA−based chemical signalling: The coordination of responses to stress in plants. *Plant Cell Environ.* **2002**, *25*, 195–210. [CrossRef]
2. Davies, W.J.; Zhang, J.H. Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 55–76. [CrossRef]
3. Huntingford, C.; Smith, D.M.; Davies, W.J.; Falk, R.; Sitchd, S.; Mercado, L.M. Combining the [ABA] and net photosynthesis−based model equations of stomatal conductance. *Ecol. Model.* **2015**, *300*, 81–88. [CrossRef]
4. Shabala, S.; White, R.G.; Djordjevic, M.A.; Ruan, Y.L.; Mathesius, U. Root−to−shoot signalling: Integration of diverse molecules, pathways and functions. *Funct. Plant Biol.* **2016**, *43*, 87–104. [CrossRef]
5. Davies, W.J.; Kudoyarova, G.; Hartung, W. Long−distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. *J. Plant Growth Regul.* **2005**, *24*, 285–295. [CrossRef]
6. Gollan, T.; Passioura, J.B.; Munns, R. Soil−water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. *Aust. J. Plant Physiol.* **1986**, *13*, 459–464.
7. Blum, A. *Plant Breeding for Water−Limited Environments*; Springer: New York, NY, USA, 2011.
8. Correia, M.J.; Rodrigues, M.L.; Ferreira, M.I.; Pereir, J.S. Diurnal change in the relationship between stomatal conductance and abscisic acid in the xylem sap of field−grown peach trees. *J. Exp. Bot.* **1997**, *48*, 1727–1736. [CrossRef]
9. Schultz, H.R. Differences in hydraulic architecture account for near isohydric and anisohydric behaviour of two field−grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ.* **2003**, *26*, 1393–1405. [CrossRef]
10. Tardieu, F.; Lafarge, T.; Simonneau, T. Stomatal control by fed or endogenous xylem ABA in sunflower: Interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant Cell Environ.* **1996**, *19*, 75–84. [CrossRef]
11. Hartung, W.; Radin, J.W.; Hendrix, D.L. Abscisic−acid movement into the apoplastic solution of water−stressed cotton leaves—Role of apoplastic pH. *Plant Physiol.* **1988**, *86*, 908–913. [CrossRef] [PubMed]
12. Mingo, D.M.; Bacon, M.A.; Davies, W.J. Non-hydraulic regulation of fruit growth in tomato plants (Lycopersicon esculentum cv. Solairo) growing in drying soil. *J. Exp. Bot.* **2003**, *54*, 1205–1212. [CrossRef] [PubMed]

13. Ali, M.; Jensen, C.R.; Mogensen, V.O.; Andersen, M.N.; Henson, I.E. Root signalling and osmotic adjustment during intermittent soil drying sustain grain yield of field grown wheat. *Field Crop Res.* **1999**, *62*, 35–52. [CrossRef]
14. Grossmann, K.; Hansen, H. Ethylene-triggered abscisic acid: A principle in plant growth regulation? *Physiol. Plant* **2001**, *113*, 9–14. [CrossRef]
15. Delker, C.; Raschke, A.; Quint, M. Auxin dynamics: The dazzling complexity of a small molecule's message. *Planta* **2008**, *227*, 929–941. [CrossRef]
16. Sofo, A.; Scopa, A.; Manfra, M.; De Nisco, M.; Tenore, G.; Troisi, J.; Di Fiori, R.; Novellino, E. *Trichoderma harzianum* strain T−22 induces changes in phytohormone levels in cherry rootstocks (*Prunus cerasus* x *P. canescens*). *Plant Growth Regul.* **2011**, *65*, 421–425. [CrossRef]
17. Fereres, E.; Soriano, M.A. Deficit irrigation for reducing agricultural water use. *J. Exp. Bot.* **2007**, *58*, 147–159. [CrossRef]
18. Ayars, J.E.; Johnson, R.S.; Phene, C.J.; Trout, T.J.; Clark, D.A.; Mead, R.M. Water use by drip irrigated late−season peaches. *Irrig. Sci.* **2003**, *22*, 187–194. [CrossRef]
19. Dos Santos, T.P.; Lopes, C.M.; Rodrigues, M.L.; de Souza, C.R.; Maroco, J.P.; Pereira, J.S.; Silva, J.R.; Chaves, M.M. Partial rootzone drying: Effects on growth and fruit quality of field−grown grapevines (*Vitis vinifera*). *Funct. Plant Biol.* **2003**, *30*, 663–671. [CrossRef]
20. Xiloyannis, C.; Montanaro, G.; Dichio, B. Irrigation in Mediterranean fruit tree orchards. In *Irrigation Systems and Practices in Challenging Environments*; Shui, L.T., Ed.; Intech: Rijeka, Croatia, 2012; pp. 321–342.
21. Buesa, I.; Badal, E.; Guerra, D.; Ballester, C.; Bonet, L.; Intrigliolo, D.S. Regulated deficit irrigation in persimmon trees (Diospyros kaki) cv. 'Rojo Brillante'. *Sci. Hortic.* **2013**, *159*, 134–142. [CrossRef]
22. Dichio, B.; Xiloyannis, C.; Sofo, A.; Montanaro, G. Effects of post−harvest regulated deficit irrigation on carbohydrate and nitrogen partitioning, yield quality and vegetative growth of peach trees. *Plant Soil* **2007**, *290*, 127–137. [CrossRef]
23. Einhorn, T.C.; Caspari, H.W.; Green, S. Total soil water content accounts for augmented ABA leaf concentration and stomatal regulation of split−rooted apple trees during heterogeneous soil drying. *J. Exp. Bot.* **2012**, *63*, 5365–5376. [CrossRef] [PubMed]
24. Romero, P.; Dodd, I.C.; Martinez-Cutillas, A. Contrasting physiological effects of partial root zone drying in field−grown grapevine (*Vitis vinifera* L. cv. Monastrell) according to total soil water availability. *J. Exp. Bot.* **2012**, *63*, 4071–4083. [CrossRef] [PubMed]
25. Lo Bianco, R.; Talluto, G.; Farina, V. Effects of partial rootzone drying and rootstock vigour on dry matter partitioning of apple trees (Malus domestica cvar Pink Lady). *J. Agric. Sci.* **2012**, *150*, 75–86. [CrossRef]
26. Mossad, A.; Farina, V.; Lo Bianco, R. Fruit Yield and Quality of 'Valencia' Orange Trees under Long−Term Partial Rootzone Drying. *Agronomy* **2020**, *10*, 164. [CrossRef]
27. Montanaro, G.; Dichio, B.; Bati, B.C.; Xiloyannis, C. Soil management affects carbon dynamics and yield in a Mediterranean peach orchard. *Agric. Ecosyst. Environ.* **2012**, *161*, 46–54. [CrossRef]
28. Fernandez, J.E.; Palomo, M.J.; Diaz-Espejo, A.; Giron, I.F. Influence of partial soil wetting on water relation parameters of the olive tree. *Agronomie* **2003**, *23*, 545–552. [CrossRef]
29. Puértolas, J.; Conesa, M.R.; Ballester, C.; Dodd, I.C. Local root abscisic acid (ABA) accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. *J. Exp. Bot.* **2015**, *66*, 2325–2334. [CrossRef]
30. Lovelli, S.; Scopa, A.; Perniola, M.; Di Tommaso, T.; Sofo, A. Abscisic acid root and leaf concentration in relation to biomass partitioning in salinized tomato plants. *J. Plant Physiol.* **2012**, *169*, 226–233. [CrossRef]
31. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, 8th ed.; Iowa State University Press: Iowa City, IA, USA, 1989; pp. 113–128.
32. Choné, X.; Van Leeuwen, C.; Dubourdieu, D.; Gaudillère, J.P. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* **2001**, *87*, 477–483. [CrossRef]
33. Garnier, E.; Berger, A. Testing water potential in peach trees as an indicator of water stress. *J. Hortic. Sci.* **1985**, *60*, 47–56. [CrossRef]
34. Remorini, D.; Massai, R. Comparison of water status indicators for young peach trees. *Irrig. Sci.* **2003**, *22*, 39–46. [CrossRef]
35. Naor, A.; Naschitz, S.; Peres, M.; Gal, Y. Responses of apple fruit size to tree water status and crop load. *Tree Physiol.* **2008**, *28*, 1255–1261. [CrossRef]

36. Xu, W.; Jia, L.; Shi, W.; Liang, J.; Zhou, F.; Li, Q.; Zhang, J. Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytol.* **2013**, *197*, 139–150. [CrossRef]
37. Brunner, I.; Herzog, C.; Dawes, M.A.; Arend, M.; Sperisen, C. How tree roots respond to drought. *Front. Plant Sci.* **2015**, *6*, 547. [CrossRef] [PubMed]
38. Martin-Vertedor, A.I.; Dodd, I.C. Root-to-shoot signalling when soil moisture is heterogeneous: Increasing the proportion of root biomass in drying soil inhibits leaf growth and increases leaf abscisic acid concentration. *Plant Cell Environ.* **2011**, *34*, 1164–1175. [CrossRef] [PubMed]
39. Saab, I.N.; Sharp, R.E. Non−hydraulic signals from maize roots in drying soil: Inhibition of leaf elongation but not stomatal conductance. *Planta* **1989**, *179*, 466–474. [CrossRef]
40. Seo, M.; Koshiba, T. Transport of ABA from the site of biosynthesis to the site of action. *J. Plant Res.* **2011**, *124*, 501–507. [CrossRef]
41. Tardieu, F.; Parent, B.; Simonneau, T. Control of leaf growth by abscisic acid: Hydraulic or non−hydraulic processes? *Plant Cell Environ.* **2010**, *33*, 636–647. [CrossRef]
42. Xiloyannis, C.; Uriu, K.; Martin, G.C. Seasonal and diurnal variations in abscisic acid, water potential, and diffusive resistance in leaves from irrigated and non−irrigated peach trees. *J. Am. Soc. Hortic. Sci.* **1980**, *105*, 412–415.
43. Trejo, C.L.; Davies, W.J. Drought−induced closure of *Phaseolus vulgaris* stomata precedes leaf water defict and any increase in xylem ABA concentration. *J. Exp. Bot.* **1991**, *42*, 1507–1516. [CrossRef]
44. Zhang, J.; Davies, W.J. Changes in the concentration of ABA in xylem sap as a function of changing soil−water status can account for changes in leaf conductance and growth. *Plant Cell Environ.* **1990**, *13*, 277–285. [CrossRef]
45. Wartinger, A.; Heilmeier, H.; Hartung, W.; Schulze, E. Daily and seasonal courses of leaf conductance and abscisic acid in the xylem sap of almond trees [*Prunus dulcis* (Miller) D. A. Webb] under desert conditions. *New Phytol.* **1990**, *116*, 581–587. [CrossRef]
46. Hartung, W.; Slovik, S. Physicochemical properties of plant−growth regulators and plant−tissues determine their distribution and redistribution—Stomatal regulation by abscisic−acid in leaves. *New Phytol.* **1991**, *119*, 361–382. [CrossRef]
47. Hartung, W.; Sauter, A.; Hose, E. Abscisic acid in the xylem: Where does it come from, where does it go to? *J. Exp. Bot.* **2002**, *53*, 27–32. [CrossRef] [PubMed]
48. Kaiser, W.M.; Hartung, W. Uptake and release of abscisic acid by isolated photoautotrophic mesophyll cells, depending on pH gradients. *Plant Physiol.* **1981**, *68*, 202–206. [CrossRef]
49. Slovik, S.; Hartung, W. Compartmental distribution and redistribution of abscisic acid in intact leaves. *Planta* **1992**, *187*, 26–36. [CrossRef]
50. Basile, B.; Bryla, D.R.; Salsman, M.L.; Marsal, J.; Cirillo, C.; Johnson, R.S.; DeJong, T.M. Growth patterns and morphology of fine roots of size−controlling and invigorating peach rootstocks. *Tree Physiol.* **2007**, *27*, 231–241. [CrossRef]
51. Nuzzo, V.; Dichio, B.; Xiloyannis, C. Canopy development and light interception in peach trees trained to transverse Y and delayed vase in the first four years after planting. *Acta Hortic.* **2002**, *592*, 405–412. [CrossRef]

Supplementary data

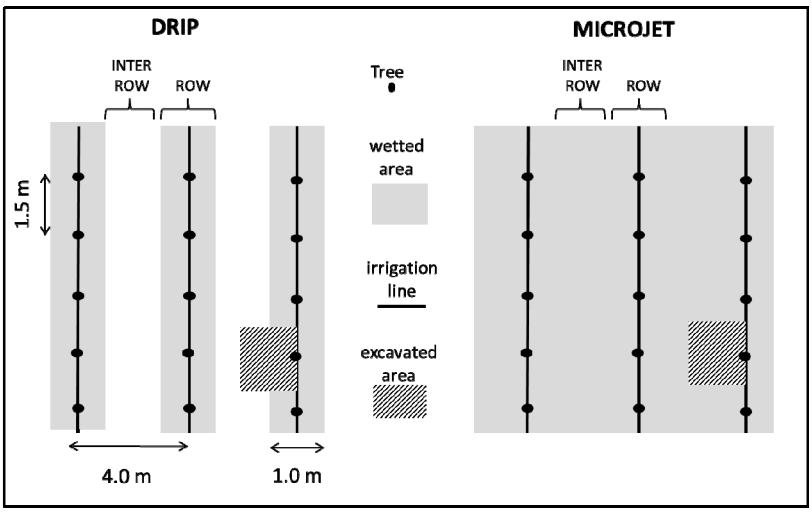

Figure S1 - Schematic representation of the drip and microjet irrigation treatments and of wetted and excavated soil area.

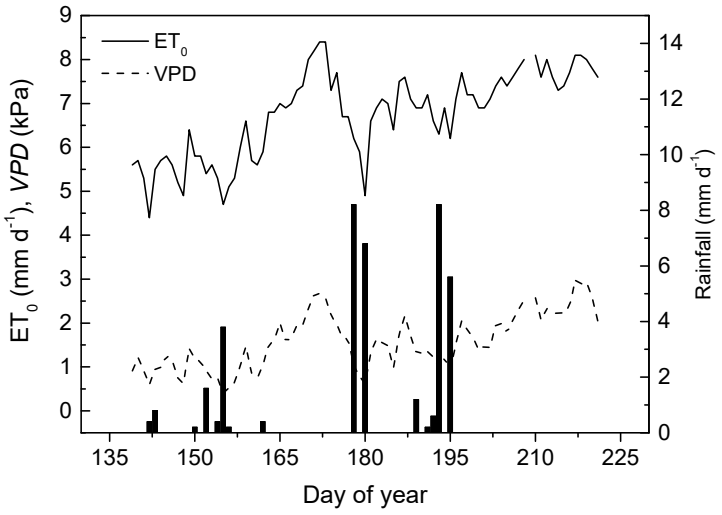

Figure S2 - Daily values of rainfall (black bars) and daily reference evapotranspiration (ET_0) (—) and vapor pressure deficit (VPD) (- - -) from DOY 141 to 220 (20th May to 8th August 2013).

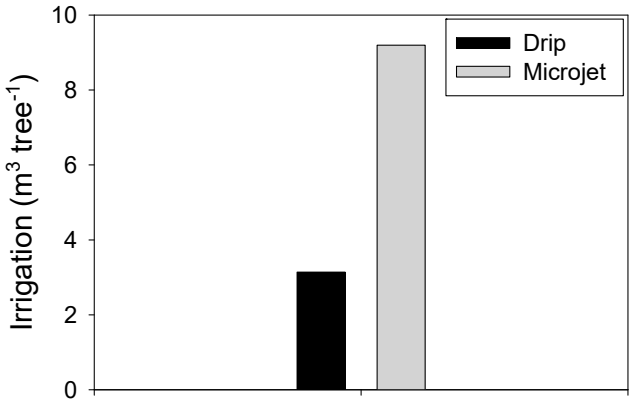

Figure S3. Irrigation volumes per tree during the experimental period (20 May-8 August 2013) for D (black column) and MJ (grey column) irrigated blocks.