Fruit and leaf sensing for continuous detection of nectarine water status

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Abstract

Continuous assessment of plant water status indicators might provide the most precise information for irrigation management and automation, as plants represent an interface between soil and atmosphere. This study investigates the relationship of plant water status to continuous fruit diameter (FD) and inverse leaf turgor pressure rates ($p_p$) in nectarine trees [Prunus persica (L.) Batsch] throughout fruit development. The influence of deficit irrigation treatments on stem ($\Psi_{stem}$) and leaf water potential, leaf relative water content, leaf hydraulic conductance and fruit growth was studied across the stages of double-sigmoidal fruit development in 'September Bright' nectarines. Fruit relative growth rate (RGR) and leaf pressure change rate (RPCR) were derived from FD and $p_p$ to represent rates of water in- and outflows in the organs, respectively. Continuous RGR and RPCR dynamics were independently and combinedly related to plant water status and environmental variables. The independent use of RGR and RPCR yielded significant associations with midday $\Psi_{stem}$, the most representative index of tree water status in anisohydric species. However, the combined use of nocturnal fruit and leaf parameters unveiled an even more significant relationship with $\Psi_{stem}$, suggesting a different fruit-to-leaf water balance in response to pronounced water deficit. In conclusion, we highlight the suitability of a multi-organ sensing approach for improved prediction of tree water status.

Keywords: fruit growth, irrigation, precision horticulture, Prunus persica (L.) Batsch, turgor pressure, water potential.
INTRODUCTION

Precision irrigation is becoming a crucial management approach for environmentally and economically sustainable fruit tree production. The vast majority of fruit crops need irrigation supply as rainfall does not match crop water requirements (Scalisi et al., 2017). In most cases of fruit crops cultivated in dry areas, rainfed agriculture is not sustainable and deficit irrigation (DI) is a reasonable tool to improve water use efficiency. Fereres and Soriano (2007) highlighted the benefits of regulated deficit irrigation (RDI) as a strategy to reduce agricultural water use. The main purpose of RDI is to reduce irrigation at specific developmental stages of the crop with no or limited effects on yield. The use of DI in different phenological stages of fruit crops started in the 80’s by Chalmers et al. (1981; 1986). Today, water supply for DI treatments is often calculated as a fraction of crop evapotranspiration (ETc) (Naor, 2006; Paço et al., 2006) or weather-based modelling crop water requirements. Additional approaches, on the other hand, rely on soil- or plant-based sensing.

Plant physiological indicators of water deficit are predominantly subjected to changes in tissue water content and status rather than to soil water dynamics (Jones, 2004; Steppe et al., 2008). Moreover, to adequately represent soil spatial variability and wetted and non-wetted zones in irrigated crops, soil-based sensing requires the use of many sensors, making this approach costly and difficult. Therefore, a continuous assessment of plant water status indicators might provide the most precise information for irrigation management and automation. The advantage of plant-based methods over soil-based techniques resides in the fact that plants are an interface between soil and atmosphere (Fernández, 2017), being in the middle of the soil-plant-atmosphere continuum (SPAC). Therefore, precise automated irrigation management is likely to be highly associated to direct or indirect measurements of plant physiological indicators.

Midday stem water potential (Ψstem) is one of the most widely used indicators of plant water status for irrigation scheduling in anisohydric plants (McCutchan and Shackel, 1992; Shackel et al., 1997; Naor, 1999). Conversely, Blanco-Cipollone et al. (2017) suggested the adoption of pre-dawn leaf water potential (Ψleaf) as a suitable parameter for irrigation scheduling in isohydric species such as grapevine. Leaf relative water content (RWC) can also be used as a water deficit indicator (Lo Bianco and Scalisi, 2017; Mossad et al., 2018), although, differently from water potential it does not give indication of water energy status (Jones, 2007). Indicators of leaf water status may not be very useful in the early detection of
plant water deficit in isohydric species (Jones, 2004), as their preventive stomatal closure preserves leaf turgor and leaf RWC.

A completely automated model for irrigation management in fruit crops is difficult to achieve, as responses to water deficit not only depend on environmental variables and soil water availability, but also on fruit tree phenology. In stone fruits (e.g. peach, nectarines, plums), tree water status and sink-source relationships differ in the three stages of the typical double sigmoidal fruit growth model (Connors, 1919; Chalmers and van den Ende, 1975), as shown in peach by DeJong and Goudriaan (1989). As a consequence, DI applied at each of the stages of peach fruit growth differently affects vegetative and fruit growth, causing changes in final fruit size and composition (Li et al., 1989a). Fruit water exchanges are driven by transpiration, phloem and xylem with different mechanisms linked to fruit growth stages (Marsal and Girona, 1997; Morandi et al., 2007a; Morandi et al., 2010a). In peach, drought induces a relatively lower reduction of fruit growth in the early stages of fruit development, compared to final stages, when cell enlargement occurs (Li et al., 1989a; Génard and Huguet, 1996).

A direct automated and continuous estimation of $\Psi_{\text{stem}}$, $\Psi_{\text{leaf}}$ or leaf RWC is not feasible yet and the use of indirect plant-based technologies might represent a viable solution for tree water status determination. Trunk-based sensing such as sap-flow methods and dendrometry have been used for irrigation scheduling in peach and several other fruit crops (Fernández, 2017). Li et al. (1989b), Simonneau et al. (1993) and Goldhamer et al. (1999) successfully associated peach tree water status to stem diameter fluctuations obtained by dendrometers built on linear variable displacement transducers (LVDTs). In addition, Conejero et al. (2007) studied peach maximum trunk daily shrinkage and sap-flow signals for irrigation scheduling, suggesting that the former represents a more sensitive indicator of plant water status. Nevertheless, the use of stem diameter variations and sap flow for irrigation scheduling is questionable. Trunk diameter fluctuations are affected by plant age and size, crop load and growth patterns (Fernández, 2017), whereas sap flow rates reflect transpiration dynamics, which are not only dependent on stomatal closure and aperture, but also on environmental variables (Jones, 2004).

The use of fruit- and leaf-based sensors to study tree water relations has also been reviewed in the literature (Jones, 2004; Fernández, 2017; Scalisi et al., 2017). The combined study of fruit and leaf water relations by continuous sensors may represent an innovative approach to determine sensitive indicators to water deficit. Changes in peach fruit water content in response to drought may be assessed with a model developed by Génard and
Huguet (1996). The most common type of fruit-based sensor used to determine when trees enter water deficit conditions are based on LVDT technologies. Lang (1990) used LVDT sensors to emphasise the role of phloem, xylem and transpiration in ruling apple fruit changes in size over time. Similar sensors were used by Morandi et al. to study vascular flows in peach (2007a; 2010a), kiwifruit (2010b) and pear (2014). Fruit growth dynamics are definitely a good indirect indicator of fruit water status (Fernandes et al., 2018), as dry matter accumulation is negligible on a daily scale (Blanke and Lenz, 1989). Fruit growth dynamics however can be influenced by growth stage and crop load. In peach, fruit water dynamics vary across the season, with maximum transpiration at fruit cell enlargement (Morandi et al., 2010a). As a consequence, the use of fruit gauges alone may not be a reliable indicator of whole plant water energy status.

Leaf-based sensing technologies mainly adopt leaf thickness sensors and pressure probes. The continuous outputs of the former were related to leaf RWC (Búrquez, 1987), although their long-term use is not feasible as they commonly injure leaves after short time (Zimmermann et al., 2008). As a consequence, recently a less invasive leaf pressure probe for the continuous determination of leaf water status (Zimmermann et al., 2008) has taken hold. These so-called leaf patch clamp pressure (LPCP) probes can be used to assess plant water status for irrigation scheduling, as they respond to leaf turgor pressure dynamics. Most of the initial studies with LPCP probes were carried out on olive (Fernández et al., 2011; Ehrenberger et al., 2012; Rodríguez-Dominguez et al., 2012; Padilla-Díaz et al., 2016) because the thick leaves of this species suit better the prolonged use of sensors. Olive is cultivated in dry or semi-dry regions with limited or no irrigation water supply. LPCP probes were also related to plant water status in other fruit crops, such as banana (Zimmermann et al., 2010), grapevine (Rüger et al., 2010), clementine (Ballester et al., 2015) and persimmon (Ballester et al., 2015; Martínez-Gimeno et al., 2017). However, as for fruit sensors, the use of LPCP probes alone can only give partial information on whole plant water status, unless many sensors are used on a tree. This is particularly due to different leaf initial conditions depending on age (especially in evergreen species) and exposure to light within the canopy. Even accepting the goodness of the data, a further need to test LPCP probes on species with thinner leaves (e.g. stone fruits) arises, as their prolonged use might damage leaf cuticle and alter readings (Scalisi et al., 2017). As mentioned above, the use of a single type of sensors can only provide partial information on tree water status. Most of C3 fruit trees exchange water with the surrounding atmosphere by means of transpiring fruit and leaves.
This study aims at investigating the relationship of plant water status to continuous fruit size and leaf turgor pressure dynamics in nectarine trees [Prunus persica (L.) Batsch] subjected to DI at each of the individual stages of fruit growth. The main hypothesis is that the combined information from fruit and leaves (i.e. the transpiring organs) provides more powerful information than individual indicators to determine plant water status on a continuous basis for adoption of precision irrigation management.

MATERIALS AND METHODS

Experimental design

The experiment was carried out in summer 2017/18 on late ripening 'September Bright' nectarine trees grafted on 'Elberta' rootstock at the research station of Agriculture Victoria, Tatura, Australia (36°26'7.2" S and 145°16'8.4" E, 113 m a.s.l.). Within the experimental site, 144 four-year-old trees trained to an open Tatura system with 4.5 × 1 m spacing (i.e. 2222 trees/ha) were selected. Trees were disposed along N-to-S oriented rows. The soil was a clay-loam and trees were regularly fertigated according to conventional protocols. Fruit thinning and summer pruning were carried out at 43 and 125 days after full bloom (DAFB), respectively.

The typical double-sigmoidal fruit growth pattern was characterized by measurements of fruit diameter in control trees at weekly intervals from shuck fall to harvest. Growth stages were divided as follows: a cell division stage (I), a pit hardening stage (II), and a cell expansion stage (III) further subdivided into two phases of about a month each, with the first (IIIa) starting when fruit cells re-establish a strong sink power after stage II, and the second being the final period of sugar accumulation and chlorophyll degradation (stage IIIb). Four different DI levels, namely 100% of crop evapotranspiration (ETc, control), 40% of ETc (DI-40), 20% of ETc (DI-20) and 0% of ETc (DI-0) were applied at each of the fruit growth stages by drip irrigation. The experimental design included six replications in a randomised complete block design, each with two tree orientations (East and West) per treatment and fruit growth stage; measurement trees were separated by buffer trees and rows. At stage IIIb the DI-40 treatment was not included, due to limited number of trees available. Canopy orientation was also included in the design, including West- and East-oriented trees of the open Tatura system. This was particularly helpful to explain different responses among trees due to light interception at different times of the day.

Meteorological data were collected using a weather station located in the experimental field. Reference evapotranspiration (ET₀) and vapour pressure deficit (VPD) were calculated
using the methods described by Allen et al. (1998). \( ET_c \) was estimated by weighing \( ET_0 \) with nectarine effective area of shade (EAS) as shown by Goodwin et al. (2006).

**Fruit size and tree water relations**

**Fruit size**

Fruit diameters were regularly measured at weekly intervals at stages I, II, IIIa and IIIb using a Calibit digital calliper (HK Horticultural Knowledge srl, Bologna, Italy). Measurements were carried out on three fruit for each irrigation treatment and canopy orientation. Data from differently oriented trees, i.e. East and West, were pooled together as fruit diameters were not significantly different at any of the stages considered.

**Water potential**

A pressure chamber (3000 Scholander Plant Water Status Consol, ICT International, Armidale, AU) was used for the measurements of \( \Psi_{stem} \) and \( \Psi_{leaf} \) according to Turner (1988). Midday \( \Psi_{stem} \) was determined at weekly intervals in all the stages of fruit growth on three leaves of the two trees (East- and West-oriented) per treatment in one of the six blocks. Daily curves from pre-dawn to 19.00h were plotted using \( \Psi_{stem} \) and \( \Psi_{leaf} \) data collected at three-hour intervals (\( \Psi_{leaf} \) was only measured at stage IIIa and IIIb of fruit growth).

**Leaf relative water content**

Leaf RWC was obtained using the method described by Barrs and Weatherley (1962). Mature leaves similar to those used for \( \Psi_{stem} \) were collected, sealed in plastic bags and transported to the laboratory for fresh weight (FW) determination. Turgid weight (TW) was obtained after immersing leaves in deionized water for 24 h at 4 °C. Subsequently, leaves were dried in an oven at 60 °C until constant weight (2-3 days) to estimate dry weight (DW). Leaf RWC was calculated as shown in Eq. 1.

\[
\text{RWC} = (\text{FW} – \text{DW}) / (\text{TW} – \text{DW}) \times 100
\]

(Eq. 1)

Leaf RWC was determined at three-hour intervals on the same days and trees as \( \Psi_{stem} \) and \( \Psi_{leaf} \) determination.

**Leaf hydraulic conductance**
A Delta-T AP4 dynamic porometer (Delta-T Devices LTD, Cambridge, UK) was used to determine leaf hydraulic conductance ($g_l$). Mid-morning (10 to 11 am) measurements of $g_l$ were undertaken at weekly intervals, whereas stage-related $g_l$ daily curves were obtained for the same days and trees as $\Psi_{stem}$, $\Psi_{leaf}$ and leaf RWC.

**Fruit diameter and leaf turgor pressure continuous sensing**

Fruit diameter (FD) was determined continuously with the LVDT-based fruit gauges described by Morandi et al. (2007b) connected to CR-1000 data loggers (Campbell scientific, Inc., Logan, US). Concurrently, leaf-mounted LPCP probes (Yara International, Oslo, NO) were used to track leaf turgor pressure dynamics using the attenuated pressure of leaf patches ($p_p$), an index which is inversely related to leaf cell turgor pressure ($p_c$), as described by Zimmermann et al. (2008). Data from both sensors were recorded at 15-minute intervals for a week period at each of the growth stages in one of the blocks within the experimental orchard. Two fruit gauges and LPCP probes were mounted on each West- and East-oriented tree, at medium canopy height and in nearby positions. Prior the actual week of measurements, a preliminary three-day comparison test between East- and West-oriented trees was carried out to verify if canopy orientation had an effect on sensors’ outputs. Data from East and West trees were compared using daily relative standard deviations (RSD), mean, sum, max and min.

Raw data obtained from fruit gauges and LPCP probes were smoothed using a 15-point convoluted spline function (Savitzky and Golay, 1964). Subsequently, FD and $p_p$ values were standardized by using z-scores (i.e. $z = (x – \text{mean}) / \text{standard deviation}$) to permit the possible comparison among fruits or leaves, respectively, which had different characteristics when the sensors were attached (i.e. fruit diameter and leaf turgor pressure). This allowed to average more sensors’ output on the same tree and compare different treatments. Furthermore, the second derivatives of fruit diameter and $p_p$ were calculated to determine fruit relative growth rate (RGR) and leaf relative pressure change rate (RPCR), as shown in Eq. 2 and 3, respectively. Second derivatives were not standardized as they are calculated based on the previous FD and $p_p$, allowing possible comparisons among outputs from different sensors.

$$\text{RGR} = \left[\ln (\text{FD}_2) – \ln (\text{FD}_1)\right] / (t_2 – t_1)$$  \hspace{1cm} (Eq. 2)

$$\text{RPCR} = \left[\ln (p_p^2) – \ln (p_p^1)\right] / (t_2 – t_1)$$  \hspace{1cm} (Eq. 3)
where \( \text{FD}_2 \) and \( \text{FD}_1 \) correspond to FD at time 2 (\( t_2 \)) and 1 (\( t_1 \)), and \( \text{p}_p \) at time 2 (\( t_2 \)) and 1 (\( t_1 \)), respectively.

Diel, diurnal and nocturnal variance of sensors’ outputs was expressed as relative standard deviation (RSD = standard deviation / mean), to allow comparison among variances of different units (i.e. FD / \( \text{p}_p \) and RGR / RPCR). In addition, also diel, diurnal and nocturnal statistical parameters from data series were calculated for the variables considered (i.e. maximum, minimum and sum values) in order to find the best predictor of midday \( \Psi \)_{stem}.

Data from sensors that either caused damage to leaves or fruit or that were displaced by strong wind were not considered in the analysis.

**Statistical analysis**

Statistical analysis was carried out using SYSTAT procedures (Systat software Inc., Chicago, US). Analysis of variance was performed when comparing irrigation treatments, canopy orientation and time factors, and, when appropriate, means were compared by Tukey’s multiple range test and honestly significant difference (HSD). Sigmaplot procedures (Systat software Inc., Chicago, US) were used for linear and multiple linear regression analyses in order to associate continuous sensors’ output to plant water status indicators.

**RESULTS AND DISCUSSION**

**Fruit developmental stages, weather conditions and crop water supply**

The typical double sigmoidal fruit development pattern was observed and Stages I, II, IIIa and IIIb lasted 35, 50, 29 and 31 days, respectively (Fig. 1).
Figure 1. Fruit diameter during each fruit growth stages of ’September Bright’ nectarines under control irrigation. Time series expressed in days after full bloom (DAFB).

Temperature (T), relative humidity (RH), $ET_0$ and VPD recorded from 27 to 173 days DAFB are shown in Figure 2. The gap in the data from 106 to 110 DAFB was due to a battery discharge. In stage I and at the beginning of stage II, frequent and abundant precipitations (Tab. 1) led to relatively low T (Fig. 2B) and high RH (Fig. 2C) (i.e. from 78 to 89 DAFB). Maximum $ET_0$ occurred in stage IIIa (Fig. 2A), driven by a combination of high T and low RH which caused a rise in VPD (Fig. 2D).
Figure 2. Daily total reference evapotranspiration ($ET_0$, A), mean temperature ($T_{\text{mean}}$, B), mean relative humidity ($RH_{\text{mean}}$, C) and mean vapour pressure deficit ($VPD_{\text{mean}}$, D) along the considered four stages of fruit growth in days after full bloom (DAFB). Missing data from 106 to 110 DAFB.

Precipitations progressively decreased towards the end of stage IIIb (Tab. 1). The highest crop water supply (CWS, i.e. rainfall + irrigation) found in stage II was likely to be due to its longer duration compared to other stages (Tab. 1).

Table 1. Total rainfall, full irrigation to control irrigated trees (FI) and crop water supply to control trees (CWS, i.e. rainfall + irrigation) at each of the fruit growth stages.

| Fruit growth stage | Duration (No. days) | Rainfall (mm) | FI (mm) | CWS (mm) |
|---------------------|---------------------|---------------|---------|----------|
| I                   | 36                  | 64            | 73      | 137      |
| II                  | 50                  | 141           | 78      | 219      |
| IIIa                | 29                  | 35            | 81      | 116      |
Fruit size and tree water relations

Fruit size

No significant difference in fruit size was found between East- and West-oriented trees, thus data from the two sides were pooled together. At stage I, fruit diameter was significantly reduced by DI at 55 DAFB, with DI-20 and DI-40 inducing similar reductions and intermediate between the control and DI-0 (Fig. 3A). At stage II, during pit hardening, fruit diameter was only slightly affected by DI treatments, and significant differences only emerged at the end of the stage between control and DI-0 trees (Fig. 3B). At stage IIIa, DI induced fruit diameter reductions similar to those at stage I, with all DI treatments showing similar reductions compared to the control. Finally, DI caused the highest reduction of fruit growth at stage IIIb (Fig. 3D). Results from DI in stages I, II, IIIa and IIIb are in line with findings in peach from Li et al. (1989a) and Génard and Huguet (1996), and in nectarines from Naor et al. (1999; 2001).

Water potential

When water potentials from East- and West-oriented trees were compared, no statistically significant differences were found, thus data from the two sides were pooled together. Daily curves of $\Psi_{\text{stem}}$ highlighted a relevant and gradual separation among irrigation treatments at solar noon measurements, except for stage II (Fig. 4), a further evidence of the
suitability of midday $\Psi_{\text{stem}}$ as an indicator of plant water deficit, as previously shown by Naor et al. (1999). The lack of an effect of DI on $\Psi_{\text{stem}}$ at stage II might be related to the abundant precipitations which occurred during this phase (Tab. 1).

Similarly, when weekly midday $\Psi_{\text{stem}}$ was considered, the effect of DI treatments increased gradually with fruit growth, reaching the most marked reductions at the end of stage IIIb (Fig 5). Even in this case, minor or no effects were found at stage II, although in the second half, decreasing precipitations (data not shown) unveiled a drop of midday $\Psi_{\text{stem}}$ in DI-0 trees (Fig. 5B). A steeper decrease of midday $\Psi_{\text{stem}}$ at stage II was also found by Fereres and Soriano in peach (2006).

Daily measurements of $\Psi_{\text{leaf}}$ carried out only in stage IIIa and IIIb (Fig. 6A and B), and concomitantly with $\Psi_{\text{stem}}$, showed typical patterns with lowest values around solar noon. As expected, $\Psi_{\text{leaf}}$ resulted in slightly lower values than $\Psi_{\text{stem}}$, in accordance with the water potential gradient along the SPAC. DI-0 trees reached the lowest $\Psi_{\text{leaf}}$ of -3.82 and -3.75 MPa in stage IIIa and IIIb, respectively (Fig. 6).

![Figure 4. Daily curves of stem water potential ($\Psi_{\text{stem}}$) at stages I (A), II (B), IIIa (C) and IIIb (D) of 'September Bright' nectarine fruit growth. Error bars represent standard errors of means (n = 6). Significant differences determined with analysis of variance and Tukey's Honest Significant Difference (HSD, P < 0.05).](image)
Figure 5. Midday stem water potential ($\Psi_{stem}$) at stages I (A), II (B), IIIa (C) and IIIb (D) of 'September Bright' nectarine fruit growth. Timeline expressed in days after full bloom (DAFB). Error bars represent standard errors of means (n = 6). Significant differences determined with analysis of variance and Tukey’s Honest Significant Difference (HSD, P < 0.05).

Figure 6. Daily curves of leaf water potential ($\Psi_{leaf}$) at stages IIIa (A) and IIIb (B) of 'September Bright' nectarine fruit growth. Error bars represent standard errors of means (n = 6). Significant differences determined with analysis of variance and Tukey’s Honest Significant Difference (HSD, P < 0.05).

Leaf relative water content
Daily curves of leaf RWC obtained from measurements carried out at all the fruit development stages and on all the irrigation treatments did not highlight differences among West- and East-oriented trees (data not shown), thus data from the two sides were pooled together. At stage I, leaf RWC varied greatly showing erratic effects of DI (Fig. 7A). At stage II, irrigation treatment and time of day had no significant effect on leaf RWC (Fig. 7B). Nevertheless, leaf RWC was found gradually lower along the irrigation treatment gradient at stage IIIa (Fig. 7C), where the maximum differences between the two extreme treatments, control and DI-0, occurred at mid-morning and mid-afternoon. Ultimately, at stage IIIb, differences among treatments were once again non-significant, except for the measurement at 19.00h (Fig. 7D). Therefore, leaf RWC cannot be considered as sensitive as $\Psi_{\text{stem}}$ and $\Psi_{\text{leaf}}$ for nectarine water status determination, mainly because the variability of RWC among leaves is high and results in non-significant effects of DI (i.e. HSD in Figure 7).

![Figure 7](image-url)  
Figure 7. Daily curves of leaf relative water content (RWC) at stages I (A), II (B), IIIa (C) and IIIb (D) of 'September Bright' nectarine fruit growth. Error bars represent standard errors of means (n = 6). Significant differences determined with analysis of variance and Tukey’s Honest Significant Difference (HSD, P < 0.05).

Leaf hydraulic conductance
Data of $g_i$ at stage I are not available due to instrument malfunctioning. At stage II, no significant differences in daily $g_i$ were found among irrigation treatments (Fig. 8A). When maximum stomatal aperture occurred (mid-morning) there was a significant influence of canopy orientation, resulting in higher $g_i$ in leaves of West-oriented trees (Fig. 8B), as they intercepted greater PAR than East trees. After noon, an overall partial closure of stomata induced a consequential reduction of $g_i$ in all the treatments. At stage IIIa, control irrigated trees expressed a $g_i$ higher than 300 mmol m$^{-2}$ s$^{-1}$ in the morning, whereas DI-0 trees barely opened their stomata (about 10 mmol m$^{-2}$ s$^{-1}$) in response to high water deficit conditions (Fig. 8C). Differently from stage I, no differences were found between West- and East-oriented trees, because measurements were done on a cloudy day (Fig. 8D). At stage IIIb, leaves of control trees had higher $g_i$ compared to DI-20 and DI-0 trees, which instead showed similar $g_i$ levels (Fig. 8E). In addition, even in the case of stage IIIb daily curve, a cloudy morning concealed the effect of canopy orientation, and the increase of photosynthetic photon flux density (PPFD) caused by the disappearance of clouds after solar noon was not sufficient to show differences between West- and East-oriented trees (Fig. 8F).
Figure 8. Daily curves of leaf hydraulic conductance ($g_l$) in control, DI-40, DI-20 and DI-0 trees at stages II (A), IIIa (C) and IIIb (E) of 'September Bright' nectarine fruit growth, and in West- and East-oriented trees (Stage II = B, IIIa = D, IIIb = F). Bars in panels B, D and F show means of photosynthetic photon flux density (PPFD) for West and East trees. Bars represent standard errors of means (irrigation treatment n = 6; canopy orientation n = 12). Significant differences determined with analysis of variance and Tukey’s Honest Significant Difference (HSD, $P < 0.05$). The HSD bars in panel B, D and F represent only differences in $g_l$, and not in PPFD (only used as a reference).

When measured at weekly intervals, $g_l$ showed no differences among irrigation treatments at stage II (Fig. 9A), whereas DI treatments reduced mid-morning stomatal aperture in the second half of stage IIIa (Fig. 9B). Only at stage IIIb, leaves from control trees consistently kept their $g_l$ higher than leaves from DI-20 and DI-0 trees (Fig. 9C). At this stage, after reaching a severe water deficit, DI-0 and DI-20 trees limited their gas exchanges
to minimal levels, and likely DI induced a reduction of phloematic flows towards fruit. Therefore, a reduction of leaf gas exchanges might partially explain the poor, non-significant increase of fruit size observed in DI-0 (Fig. 3D). Overall, $g_l$ data at mid-morning were found to be representative indicator of plant water deficit, as that is the time of highest leaf transpiration and maximum evidence of partial stomatal closure in response to water deficit.

![Image](https://via.placeholder.com/150)

**Figure 9.** Mid-morning leaf hydraulic conductance ($g_l$) at stages II (A), IIIa (C) and IIIb (D) of 'September Bright' nectarine fruit growth. Error bars represent standard errors of means ($n = 6$). Significant differences determined with analysis of variance and Tukey’s Honest Significant Difference (HSD, $P < 0.05$).

**The interdependency of plant water status indicators**

Among the others, $\Psi_{stem}$ can be considered as the most sensitive indicator of plant water status in nectarines, and it is strictly related to other water status indices along the SPAC (e.g. $\Psi_{leaf}$ and external VPD) and to the regulation of stomatal opening, expressed in terms of $g_l$. Leaf RWC has also been linked to $\Psi_{stem}$ as shown by Koide et al. (1989), although results of this study were not always in line. Indeed, leaf RWC was not found to be a sensitive measurement to highlight differences among irrigation treatments, especially at stage I, II and IIIb of fruit development (Fig. 7A, B and D). In our case, the strongest association between leaf RWC and $\Psi_{stem}$ occurred at pre-dawn, when water potential and water content were in equilibrium (data not shown).

The combined interdependency of VPD, $\Psi_{leaf}$, $g_l$ and leaf RWC with $\Psi_{stem}$ was tested analysing data extrapolated from daily curves from all the fruit growth stages. Data were pooled together and associated to $\Psi_{stem}$ through a multiple linear regression model. Stomatal aperture and closure dynamics are known to be regulated by $\Psi_{leaf}$ among other factors, which
in turn is influenced by VPD and strictly related to \( \Psi_{\text{stem}} \). Leaf RWC is then adjusted responding to water potential gradients along the SPAC. Therefore, we expected to find the strongest association of \( \Psi_{\text{stem}} \) with \( \Psi_{\text{leaf}} \), followed by decreasingly tight associations with \( g_l \), VPD and leaf RWC, respectively. However, leaf RWC resulted to be non-significant in a first backward stepwise regression model (\( P = 0.98 \)), and it was excluded from the final outcome. Minor leaf RWC changes on a daily scale (Fig. 7) may explain the absence of a relationship with \( \Psi_{\text{stem}} \). In the obtained multiple linear regression model, \( \Psi_{\text{stem}} \) was predicted from a linear combination of \( \Psi_{\text{leaf}}, g_l \) and VPD (\( R^2 = 0.867, p < 0.001, \text{S.E.} = 0.240 \)), as shown in Eq. 4.

\[
\Psi_{\text{stem}} = -0.311 + (0.882 \times \Psi_{\text{leaf}}) + (0.004 \times g_l) + (0.077 \times \text{VPD}) \quad \text{(Eq. 4)}
\]

Our results are in line with findings in nectarines and other woody species (Naor, 1998), where \( \Psi_{\text{stem}} \) was found to be related to leaf stomatal conductance (\( g_s \)) and \( \Psi_{\text{leaf}} \).

**Fruit diameter and leaf turgor pressure continuous sensing**

The preliminary trial on FD, \( p_p \), RGR and RPCR of East- and West-oriented trees did not show any significant effect of canopy orientation. As a consequence, at each of the fruit growth stages, FD and \( p_p \) data, as well as their derivatives (i.e. RGR and RPCR), from East- and West-oriented trees were pooled together. In control trees, FD showed an expected nocturnal increase with a diurnal lag phase (Fig. 10A). In the warmest hours of the day, \( p_p \) increased (Fig. 10B), being the inverse of \( p_c \), as leaf turgor pressure was lost.
Figure 10. Diel trends of fruit diameter (FD, n = 3) and fruit relative growth rate (RGR, n = 3) (A), attenuated pressure of leaf patches (p$_p$) and leaf relative pressure change rate (RPCR) (B) in control irrigated trees at stage I (51 DAFB) of 'September Bright' nectarine fruit growth.

Initially, FD and p$_p$ values, correspondent to the time of spot measurements of $\Psi_{stem}$, $\Psi_{leaf}$, $g_l$ and leaf RWC from daily curves, were considered to determine whether any significant linear relationships occurred. Pearson’s correlation analyses emphasised in most cases, no significance at all between FD and the water status indices, except for the association between FD and $\Psi_{leaf}$ with a low correlation coefficient (Tab. 2). The inverse relationships with the highest correlation coefficients were found between p$_p$ and leaf water status indices. The highest correlation coefficient was found between p$_p$ and $\Psi_{leaf}$ due to the high influence of leaf turgor pressure on the total $\Psi_{leaf}$. The use of FD and p$_p$ per se in association with plant water status indices is likely to hide information as there is an intrinsic delay in the adjustment of water in tissue in response to plant water deficit. Therefore, RGR and RPCR can be used to smooth delay of fruit and leaf responses to water deficit over time. Besides, the use of continuous data from leaves or fruit alone might not provide appropriate information on plant water status. When considered in isolation, data from fruit diameter changes are influenced by fruit development stage and fruit growth, while data of leaf turgor pressure would ignore water balance in the other main organs capable of transpiration. As a
consequence, the association of RGR and RPCR dynamics can highlight leaf-to-fruit water exchanges which might reflect more precisely plant water status.

Table 2. Pearson’s correlation coefficients for fruit diameter (FD) and attenuated leaf patch clamp pressure ($p_p$) vs plant water status (PWS) indicators: stem water potential ($\Psi_{stem}$), leaf water potential ($\Psi_{leaf}$), leaf hydraulic conductance ($g_l$) and leaf relative water content (RWC).

| PWS indicator | FD (z-scores) | P-value | n  | $p_p$ (z-scores) | P-value | n  |
|---------------|---------------|---------|----|-----------------|---------|----|
| $\Psi_{stem}$ (MPa) | -0.103 | 0.184 | 168 | -0.320 | <0.001 | 180 |
| $\Psi_{leaf}$ (MPa) | -0.296 | 0.009 | 78 | -0.645 | <0.001 | 84 |
| RWC (%) | -0.156 | 0.066 | 140 | -0.442 | <0.001 | 150 |
| $g_l$ (mmol m$^{-2}$ s$^{-1}$) | 0.183 | 0.090 | 87 | 0.186 | 0.067 | 97 |

Subsequently, data of diel relationships (i.e. $p_p$ vs FD and RPCR vs RGR) at 15-min intervals were plotted for a clear sky day at each stage of fruit development. Scatter plots in Figure 11 highlight anti-clockwise hysteretic relationships between RPCR and RGR. Similar trends were found for $p_p$ vs FD associations (data not shown). Hysteresis among sensors’ outputs and/or plant water status is common, especially when trunk or leaf indicators are considered (e.g. sap flow density, hydraulic conductance, diameter variations, $\Psi_{leaf}$, transpiration, etc.), and has been widely documented (Brough et al., 1986; Cruiziat et al., 1989; Granier et al., 1989; Ameglio and Cruiziat, 1992; Tognetti et al., 1996; Fernández, 2017). The hysteretic behaviour was observed in all the fruit developmental stages, although it showed different patterns (Fig. 11). At stage I, there was a gradual increase of the hysteretic loop area as irrigation volume decreased, reaching its maximum size in the DI-0 treatment (Fig. 11A).
Figure 11. Scatter plots of diel leaf relative pressure change rate (RPCR) and fruit relative growth rate (RGR) in control, DI-40, DI-20 and DI-0 at stages I (A), II (B), IIIa (C) and IIIb (D) of 'September Bright' nectarine fruit growth. Midday $\Psi_{stem}$ for each of the days considered is reported in its relative panel. Axis scales are equal in all panels and consequently omitted.

Nevertheless, a similar trend in loop area with higher levels of DI was not observed in the other stages, suggesting stage-dependent mechanisms of water regulation in fruits and leaves. In addition, the generally low midday $\Psi_{stem}$ at stage IIIa and IIIb (i.e. always $< -2.00$ MPa) may have altered the hysteretic patterns. Hysteresis is likely to be caused by both a lag in tissue water de- and re-hydration, and nocturnal/diurnal inverted pattern of the RPCR to RGR association. Consequently, diel RGR and RPCR trends were firstly considered alone and then subdivided into diurnal (7.00 to 19.45h) and nocturnal (20.00 to 6.45h) data, to investigate associations with midday $\Psi_{stem}$. The use of RGR and RPCR was favoured over FD and $p_p$, as the former yielded the tightest associations with midday $\Psi_{stem}$. Diel, diurnal and nocturnal RGR and RPCR parameters (i.e. RSD, maximum, minimum, sum) from all the irrigation treatments were pooled together and their means were linearly regressed with midday $\Psi_{stem}$. Among all the significant (P < 0.05) regression models obtained, the highest $R^2$
were found when nocturnal maximum RGR (MAX\textsubscript{RGR}) (Fig. 12A) and minimum diel RPCR (MIN\textsubscript{RPCR}) (Fig. 12B) were related to midday $\Psi_{stem}$. The non-linear model in Figure 12A can be explained with the fact that a limited water deficit is needed for maximum fruit cell expansion due to rehydration (i.e. peak at -1.56 MPa). Oppositely, at $\Psi_{stem}$ near -1.00 MPa fruit cell turgor is higher and less water is drawn from nearby organs. When $\Psi_{stem}$ reaches particularly low levels (~ -3.50 MPa) maximum RGR tends to zero.

The linear relationship between $\Psi_{stem}$ and MIN\textsubscript{RPCR} showed a loose but direct association (Fig. 12B), in contrast with findings in olive where Marino et al. (2016), instead, found an inverse linear relationship. In our case, even the linear regression between $p_{p}$ (the indicator used by Marino et al., 2016), rather than RPCR, and midday $\Psi_{stem}$ resulted in a direct
relationship, although with a lower $R^2$ (0.247) than the former (data not shown). The inverse relationship found by Marino et al. (2016) in olive was expected as $p_p$ is the inverse of turgor pressure, which is instead directly related to $\Psi_{stem}$. In our case, $\text{MIN}_{RPCR}$ indicates the speed at which dehydrating leaves draw water from nearby tissues. Therefore, the direct relationship between $\text{MIN}_{RPCR}$ and $\Psi_{stem}$ shows that such instantaneous water pulling force increases with water deficit, allowing leaves to maintain minimum hydration and escape desiccation and death. Indeed, a $\Psi_{stem} < 3.00$ MPa could be fatal for nectarine trees if a drought avoidance mechanism is not activated. On the other hand, olive can easily tolerate leaf dehydration at similar levels of $\Psi_{stem}$.

Insights from figures 14 and 15 suggested that ratios of RGR to RPCR might be better indicators of midday $\Psi_{stem}$, by combining fruit and leaf water relations. More specifically, the changes in hysteretic patterns (Fig. 11) indicated that RGR/RPCR variance may be strictly related to midday $\Psi_{stem}$ variations, as the shape of the loop changed along with increasing water deficit. However, hystereses were also likely to be shaped by intrinsic parameters of diel, diurnal and nocturnal variations, such as maximum, minimum and sum. As a consequence, linear regression models considered RGR-to-RPCR ratios for all these parameters regressed vs midday $\Psi_{stem}$. The only two linear models with $R^2 > 0.3$ were found for nocturnal data using the $\text{RSD}_{RGR}/\text{RSD}_{RPCR}$ ($R^2 = 0.346$) and $\text{MAX}_{RGR}/\text{MAX}_{RPCR}$ ($R^2 = 0.318$) ratios. The latter relationship was mostly derived from the significant association found in Figure 12A, as the response to midday $\Psi_{stem}$ had a similar peak trend, but with a lower $R^2$ (0.405). Therefore, the $\text{MAX}_{RGR}/\text{MAX}_{RPCR}$ ratio was discarded.

Finally, stepping forward to the strongest association with midday $\Psi_{stem}$, the scatter plot showed an inverse non-linear association (Fig. 13C), suggesting that the model might be both composed by a linear phase at higher values of $\Psi_{stem}$ and by an exponential phase at lower $\Psi_{stem}$. In accordance with our hypothesis, the diurnal regression tended to show an opposite trend, although no significant association was found (Fig. 13B). The diel regression reflected the unpredictable hysteretic behaviour seen in Figure 11, resulting in the weakest, non-significant association (Fig. 13A).
Figure 13. Diel (A), diurnal (B) and nocturnal ratios (C) of relative standard deviations of fruit relative growth rate (RSD\textsubscript{RGR}) and leaf relative pressure change rate (RSD\textsubscript{RPCR}) vs midday $\Psi_{stem}$. Expo-linear model in panel C: $\frac{\text{RSD}_{RGR}}{\text{RSD}_{RPCR}} = -0.07 + 2.88 \times 10^{-7} \times \exp(-3.89 \times \Psi_{stem}) - 0.12 \times \Psi_{stem}$, $R^2 = 0.650$, $P < 0.001$. Data from all fruit growth stages included in the model.

The association of nocturnal $\frac{\text{RSD}_{RGR}}{\text{RSD}_{RPCR}}$ to $\Psi_{stem}$ (Fig. 13C) shifted from linear to exponential at midday $\Psi_{stem} \approx -2.3$ MPa, suggesting that this water deficit level might be identified as a threshold under which late ripening 'September Bright' nectarine trees are significantly affected by drought. Below the level of -2.3 MPa the RSD of nocturnal fruit growth increases with respect to the one of leaf turgor pressure. For instance, the slight decrease in fruit diameter occurring between 154 and 161 DAFB in DI-0 trees (Fig. 3D) induces an increase in nocturnal $\text{RSD}_{RGR}$ while $\text{RSD}_{RPCR}$ does not change, generating the observed increase of $\frac{\text{RSD}_{RGR}}{\text{RSD}_{RPCR}}$. At stage III, peach and nectarine stomata become dysfunctional (Chalmers et al., 1983) and high transpiration rates can overcome level of phloem and xylem inflows in fruits (Lescourret et al., 2001; Morandi et al., 2007a). This phenomenon generates particularly low fruit water potential and causes an increase in water
potential difference between leaves and fruit (McFadyen et al., 1996), as also found in olive by Fernandes et al. (2018). Therefore, the different regulation of water balance in fruits and leaves may provide a very useful parameter for real-time and continuous monitoring of plant water status.

The identified stage-independent threshold of midday $\Psi_{stem} = -2.3$ MPa might be used for irrigation management in commercial 'September Bright' nectarine orchards under environmental conditions similar to the ones of this study. However, it probably would not be effective at stage I, as trees exposed to DI at this stage never reached such low levels of $\Psi_{stem}$, despite yielding fruit with significantly lower final size compared to control irrigated trees (i.e. average fruit diameter at harvest equal to 53.3 mm ± 0.44 vs 58.6 mm ± 0.81 for DI and control, respectively). Hence, it is legitimate to think that, to some extent, trees adjust to water deficit levels throughout the season and different $\Psi_{stem}$ thresholds should be considered at each fruit growth stage for irrigation management.

CONCLUSIONS

Overall, this work highlights the appropriateness of a multi-organ, fruit-to-leaf sensing approach for the quality of continuous monitoring of tree water status. On one side, the leaf sensing approach guarantees a fast and responsive signal based on leaf turgor pressure which represents a pre-alarm forecast for irrigation management; on the other side, continuous fruit size sensing provides the exact information on the time-lag and plant dehydration level to which deficit irrigation can be pushed before fruit growth and yield are significantly affected. Both together, leaf and fruit sensing provide a powerful and reliable tool that is not influenced by the fruit development stage and that can be continuously used to detect plant water status and irrigation thresholds. At this regard, further efforts should be made to develop new fruit and leaf sensing technologies which reduce the likelihood to damage organs during the period of data collection. Further investigations need also to be carried out considering fruit-to-leaf water balance, perhaps promoting models which considers together nocturnal to diurnal shift within the diel hysteresis of fruit growth vs leaf turgor pressure, and the lag in time characterising the hysteretic loop.

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