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Assessment of leaf water potential and stomatal conductance as early signs of stress in young hazelnut tree in Willamette valley

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ABSTRACT

Corylus avellana L. is a species highly susceptible to water stresses caused by vapor pressure deficit and high temperature. Under such conditions, transpiration is strongly constrained even with good soil water availability. This is due to ineffective drought resistance mechanisms and indicates the need to identify early indicators of plant stress that are easy to measure, effective and efficient. This research explored the possibility of using stomatal conductance and leaf water potentials as early indicators of stress. For this purpose, an experiment was set up in a commercial 5-year-old *Corylus avellana* L. var. `McDonald` orchard located in Willamette valley. The experimental designed featured irrigated and rainfed trees, and potential stress indicators were monitored at different times of the day in canopy sections aligned to cardinal directions.

Results show that hazelnut trees rapidly reduced leaf stomatal conductance when the vapor pressure deficit increased to 2 and 2.5 kPa during the diurnal cycle in both irrigated and rainfed trees, even with good water availability. This suggests leaf stomatal conductance can be an efficient and effective early indicator of stress. In addition, results suggest that stomatal conductance should be measured on leaves on the west and north aspects of the canopy, where they showed lowest and highest values respectively. Leaf and stem water potential values increased during the measurement period and show a strong correlation, but their mean values do not show statistically significant differences between treatments. In Willamette valley conditions, stomatal conductance provided earlier indication of stress than water potential. The results obtained are of methodological importance for the future design of experimental plans.

1. Introduction

The hazelnut tree (*Corlyus avellana* L.) is a shrubby plant that grows native in deciduous forests of Europe and Western Asia as an understory species preferring upland areas shaded by tall trees (Del Favero, 2004; Kull and Niinemets, 1993; Kirchlechner, 1900). As an important tree nut worldwide, *C. avellana*, is cultivated in monocultural orchards in Mediterranean climates, including environments characterized by experience extremes of drought and heat during the growing season (Di Lena et al., 2022). Demand for increased production of hazelnuts has resulted in further expansion of plantings in less suitable environments. (Spiegel et al., 2020). Climate change is adding to the complexity with increases in mean temperatures and reduced rainfall in some production areas (IPCC, 2013; An et al., 2020). Recently, Vinci et al. (2023) showed how temperatures and water requirements have increased significantly in hazelnut orchards cultivated in central Italy over the past 47 years, resulting in decreased of chilling accumulation.To optimize sustainable production of hazelnuts, it is critical to understand and manage drought stress. Research indicates that even moderate vapor pressure deficit (VPD) has a strong influence on drought stress and stomatal activity of hazelnuts which in turn are likely to represent a major limitation on production. *Corylus avellana* L. is believed to be a water-saving species that takes advantage of low VPD values to optimize stomatal activity and carbon uptake (Trotter 1951; Baldwin et al., 2003; Pasqualotto et al., 2021). Practice of grafting help to tolerate climatic variations during the growing season in terms of carbon allocation and optimization of stomatal activity (Portarena et al., 2021). Hazelnut shows a conservative isohydric tendencies under stress conditions, with stomata closing early

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in response to increasing VPD, prioritizing water use efficiency at the cost of carbon assimilation for biomass production. Several authors consider *C. avellana* to be highly sensitive to water stress with low capacity for stomatal control (Girona et al., 1994; Cristofori et al., 2014). A study conducted on three different cultivars of *C. avellana*, including Tonda Gentile delle Langhe, Tonda Romana and Tonda di Giffoni, which are typical varieties representing different climatic conditions, indicates that stomatal conductance, and thus leaf gaseous exchanges, are mainly affected by VPD (Cincera et al., 2019). Photosynthesis decreased at high levels of VPD, even if the limiting factor was increased temperature. Stomata in cultivar Tonda Gentile delle Langhe were particularly sensitive to VPD, suggesting that it is poorly adapted to more xeric environments outside of the more moderate environment of its native area, and demonstrating that VPD sensitivity can differ between hazelnut cultivars. Schulze and Küppers (1979) affirmed that with severe VPD there is sudden stomatal closure and decrease assimilation rate in *C. avellana*. Tombesi et al. (1994) state that due to stomatal dysfunction in *C. avellana*, there is high transpiration loss during water stress conditions. The susceptibility of hazelnut to severe VPD is particularly evident in the summer period with dry, hot winds, which cause leaf edge burning (Baldwin et al., 2003). Different strategy could be use to limit leaf damage. Use of kaolin spray reduces leaf temperature without interfering on photosynthetic activity, stomatal conductance, and water use efficiency (Luciani et al., 2019a). Photosynthetic activity could be enhanced, using arbuscular mycorrhizal such as G. iranicum, during spring. The latter promotes leaf physiological performances during the summer and post-harvest until leaf fall (Luciani et al., 2019b) contributing to support roots growth during the fall season (Tombesi, 1979).

Pasqualotto et al. (2018), found that in hazelnut the diurnal trends of stomatal opening reached a maximum very early in the morning (around 9 a.m.) and then strongly declined when the vapor pressure deficit exceeds 1 kPa, even when soil water is not a limiting factor. The transpiration process is restricted at certain VPD levels in *C. avellana*, even with optimal soil water content. Consequently, low relative air humidity, often combined with high temperatures and in the presence of intense solar radiation, reduces leaf transpiration and impacts the thermoregulation effect, explaining the severe burn damage to photosynthetic tissues under these conditions (Pasqualotto et al., 2016).

Pasqualotto et al. (2021) compared the conductance at the canopy level of commercial *C. avellana* orchards to VPD in different countries found that canopy conductance was strongly dependent on the VPD. Maximum canopy conductance occurred within a VPD range of 0.35–0.57 kPa with a mean VPD of 0.57 kPa. Although optimal value of VPD conditions occurred on average at 1 kPa, there was high variability among sites based on climatic conditions indicating that plants had acclimated to the more extreme sites, modifying their response to climate. In addition, at different sites, the response of the tree canopy in terms of maximum conductance was influenced by the value of Leaf Area Index (LAI), which is a function of tree spacing and training shape. Orchards with higher LAI had higher maximum stomatal conductance values associated with higher yields (Pasqualotto et al., 2021).

Aside from stomatal conductance, leaf water potential is an important index to evaluate the water status of plants (Jones, 1990; Koide et al., 1989). Knipfer et al. (2020), working in almond, walnut, and grapevine showed that the relationship between dawn and midday water potentials is predictive of stomatal closure and turgor loss under drought conditions. Shackel et al. (1997) found that daily changes in stem water potential in fruit trees under well-watered conditions correlate well with midday VPD, and that midday stem water potential is a reliable method for defining plant stress state. In *C. avellana*, the relationship between water potential and stomatal closure or reduction of gas exchange activity has been little investigated. Diurnal measurements of leaf water potential decreased linearly up to values of 1 kPa of VPD. However, when VPD exceeded values of 1 kPa no significant relationship between leaf water potential and VPD was shown, demonstrating an ability of the plant to maintain leaf water potential above a minimum threshold value, below which xylem cavitation could occur (Hogg et al., 2000). Furthermore, increased leaf mass area and leaf tissue density contributed to water stress resistance through increased resistance to physical damage caused by desiccation as found in hazelnut saplings (Catoni et al., 2017). Hogg et al. (2000) found an inverse relationship between stomatal conductance and vapor pressure deficit in beaked hazelnut (*C. cornuta* Marsh.) in the boreal forest, even when soil water was not a limiting factor.

However, predawn leaf water potential value could be considered the best indication of the water status at the crop level (Chastain et al., 2014). At that time there is no transpiration, and the water potential is in equilibrium throughout the plant, so it can be considered equivalent to the soil matric potential sensed by the roots (Amèglio et al., 1994). In grapevine, leaf water potential at predawn, leaf water potential at midday, and stem water potential at midday were evaluated as equally representative evaluation methods of plant water status, showing highly significant linear relationships (R^2 ranging from 0.85 to 0.92) with soil water content and leaf gas exchange, particularly for midday measurements of leaf gas exchange (Williams and Araujo, 2002). In contrast, in almond, walnut, and grapevine, a nonlinear relationship was shown between leaf water potential at predawn and midday (Knipfer et al., 2020) while a low correlation was found between leaf water potential and stem water potential in apple (Naor et al., 1995). Productivity and gas exchanges in the hazelnut cultivar Tonda di Giffoni, showed no significant changes up to threshold values of midday stem water potential between −7 and −10 bar, while threshold values below −13 to −16 bar showed improved water use efficiency but lower productivity and gas exchanges (Ortega-Farias et al., 2020). However, it remains unknown if plant water potential thresholds corresponding to stomata closure, and loss of leaf turgor can be predicted directly from plant water potential measurements. This would certainly allow for a more time-efficient and less labor-intensive evaluation of both physiological responses (Meinzer et al., 2016).

Water shortage, by reducing photosynthetic activity, results in a decrease in water use efficiency. The latter decreases during the day and remains with constant values up to 60% of available water, after which it drops to lower values. Consequently, to maintain high foliar assimilation, soil water content must be above 60–65% of field capacity (Tombesi et al., 1994). Several papers indicate watering onset values between 50% (Cristofori et al., 2014) and 70% of available soil water, determined by gravimetric method. Irrigation for maximizing water conservation is based on direct measurement methods of soil and plant water status. The water status of plants and soils is often defined in terms of water potential (Levin and Nackely, 2021). Understanding of physiological limits of a crop provides crucial information on crop survival and performance and can help optimize irrigation. This study aimed to:

1) Study the ecophysiological response in irrigated and rainfed hazelnut trees during the 2022 summer season through measurements of stomatal conductance and plant water potential as early indicators of drought stress;
2) measure predawn leaf water potential, midday leaf water potential, and midday stem water potential to test the hypothesis that these are equally representative assessment methods of plant water status;
3) identify canopy exposures, according to the four cardinal points, that best express the plant's physiological response.

2. Material and methods

2.1. Experimental design

The experiment was conducted in a commercial hazelnut orchard in the Willamette Valley located at latitude of 44°26′46.23 N 123°18′21.23 W, near the city of Corvallis, in Benton County, Oregon (USA), in July and August 2022 Due to continuous rainfall, it was not possible to start the trial before July.

The soil was a Woodburn silt loam(Soil Survey USDA, 2009) (Table S1)) rated 65 on a 0–75 scale for suitability to hazelnut production based on texture, drainage, and depth of native productivity according to Olsen (2013). The Willamette valley area is characterized by Mediterranean-type climate with warm, dry summers and mild, but wet winters. The mean annual temperature is approximately 10° to 13°C and receives consistent winter precipitation due to the westerly flow of Pacific storms. The mean annual precipitation is 1228 mm, ranging from 900 mm to 1600 mm in the mountainous foothills (Griffith, 2010).

The experimental design featured three factors of variability: irrigation, leaf position in the canopy (aspect or side), and time of day. Irrigation featured two levels: irrigated and rainfed trees. Irrigation in irrigated trees was triggered when soil water potential sensors at 0–30 cm depth detected values between 80 and 90 kPa, as suggested by Irmak et al. (2016); Canopy aspect featured four levels: south, north, east, and west. Measurements were taken on leaf on five randomly replicated trees per treatment rainfed. Time of day was included for selected variables with levels specified in the following paragraphsNo rainfall was recorded during the experimental hazelnut trial, but soil matric potential was maintained near field capacity by irrigation. Rainfed plants were allowed to deplete water in the soil. The orchard was comprised of 5-year-old 'McDonald' hazelnut trees (Mehlenbacher et al., 2016). The trees were trained on single trunk and planted 6 m between row and 3 m on the row, with a density of 555 trees per ha^{-1} and north-south row orientation. Irrigation was applied between July 26 and Aug 21 (Table 1) with a drip system with one line per tree; drippers were placed 0.6 m apart, thus there were five drippers per tree. The theoretical flow rate was of 1.58 liter per hour and actual of flow rate 1.63 liter per hour. Ten random trees (Fig. 1) and plant characteristics were selected from an area within the orchard. Tree trunk diameter was normally distributed around a mean of 7 cm (Fig. 2), which was used to select trees with a 7 cm mean trunk diameter. For tree selection, trunk diameter was measured, by caliper, at 40 cm above the soil line, of 13 trees in each row from 32nd through 39th, for a total of 104 trees, after discarding the first 4 border plants. This procedure allowed selection of five irrigated and five rainfed trees (green and yellow circles, respectively in Fig. 1) with statistically similar trunk size. These latter were deprived of irrigation by cutting the irrigation tubing at adjacent trees (indicated as yellow cross in Fig. 1) and splicing in blank tubing.

Environmental variables were collected from the weather station nearest to the experimental field, located in Corvallis (https://www.usbr.gov/pn/agrimet/agrimetmap/crvoda.html). In addition, throughout the study period air temperature, relative air humidity and dew point were recorded every five minutes by a portable data logger (Kestrel DROP D2®).

2.2. Measurements

2.2.1. Soil water status

Soil water status was monitored with resistance-type sensors, that measuring soil matric potential, Watermark® Granular Matrix model 200SS (Irrometer, Co., Riverside, California). The range of matric potential, measured by portable handheld meter is from 0 to 200 kPa, where 0 means saturated soil and 199 dry soil (www.irrometer.com). The sensors were tested before installation, following the manual's procedure. They were tested through two cycles of wetting and drying, soaking in water overnight and next day air drying. After sensors soaked in water, the handheld meter read values between 0 and 5 kPa and after air drying the reading had to greater than 150 kPa. *Re* soaking the sensor back in water, readings had to return below 5 kPa within 2 min. Just prior installation in the soil, the sensors were soaked in water for 30 min because wetting improves the response time by removing the air inside them (Irmak et al., 2016). The sensor's cables were put into PVC pipe which facilitates installation to the desired depth in soil. The depth of sensors was chosen considering the root zone depths which were between 40 and 60 cm. The sensors were placed in the root zone at three depths, 30, 60 and 90 cm at 20 cm away from the trunk in a triangular pattern, using a slide hammer to pilot holes in the soil. Sensors were installed for each selected irrigated or rainfed tree in the trial. Readings by portable meter were punctual and were taken at least once every other day from July 1 to August 25, 2022.

Table 1
Watering days of July and August.

Date watering	Irrigation hours	Liter per tree	m^3/ha
26-Jul	16	126.4	70.2
29-Jul	6	47.4	26.3
30-Jul	10	79	43.8
6-Aug	10	79	43.8
13-Aug	8	63.2	35.1
17-Aug	5	39.5	21.9
21-Aug	12	94.8	52.6
Sum		**529.3**	**293.8**
Mean		**75.6**	**42.0**

2.2.2. Leaf stomatal conductance

Stomatal conductance of leaves was measured using portable automatic diffusion porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK). Measurements were made on fully expanded, mature and well-exposed leaves of the ten trees, measuring one leaf for each cardinal direction. Data collection started on July 26th with four measurements per day: in the morning between 8 and 9 am (a), between 10 and 11 am (b), between 12 and 1 pm (c) and from 2 pm onward. All the readings were accomplished in around 45 min to minimize variation of water status of leaves. The porometer was calibrated using the calibration leaf, before each measurement cycle, or otherwise whenever the display indicated the need for calibration, as recommended by the manual. Three measurement cycles were carried out: the first at the start of irrigation from July 26 to 30, followed by the second measurement cycle from August 4 to 6, and finally the third cycle from August 14 to 18.

2.2.3. Plant water status: leaf and stem water potential

The measurement of plant water status included measurement of Predawn leaf water potential (Ψ_{PD}), Midday leaf water potential (Ψ_{ML}) and Midday stem water potential (Ψ_{MS}). Plant water potential readings were conducted according to the procedures of Padgett-Johnson et al. (2000) and Koide et al. (1989), using a pressure chamber (model 3005H07G4P40, Soil Moisture Equipment, Santa Barbara, Calif.). Ψ_{PD} measurements started at 4:00 am and were finished before sunrise, which started on July 1, at 5.32 am; Ψ_{ML} and Ψ_{MS} occurred between 12:00 pm and 15:30 pm, Pacific Daylight Time, starting with Ψ_{ML} and after Ψ_{MS}, considering that solar midday occurred at 13.15 pm (https://www.timeanddate.com/sun/@7173236). Leaves chosen for Ψ_{PD}, Ψ_{ML} and Ψ_{MS} were fully expanded, mature and well-exposed leaves, taken from the same bough. At 10 am, for midday measurements only, leaves for determination of Ψ_{MS} were enclosed in aluminum bags and quickly sealed with zipper closure for 2 h prior to excising. Petioles were cut by razor blade and the time between leaf excision and chamber pressurization was generally <10 to 15 s. For each tree, all measurements were conducted on four leaves, each for a cardinal direction. Thus, in total, 20 leaves per irrigated treatment and 20 leaves per rainfed trees. Measurements of Ψ_{ML} and Ψ_{MS} started on July 13th and were conducted on the same days, while for five days all three potentials were conducted including Ψ_{PD} (Table S2).

3. Statistical analysis

The experimental data were analyzed statistically by R software version 4.2.1 (R Core Development Team, 2022). Data were subjected to

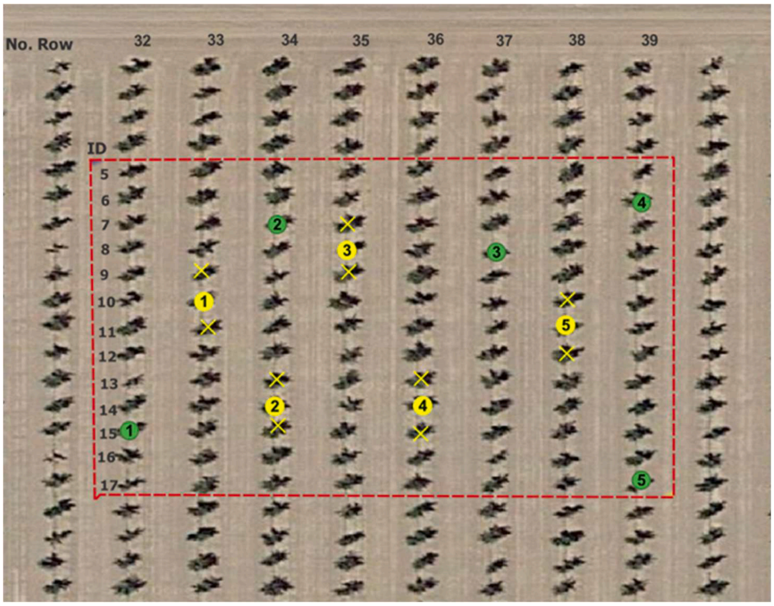

Fig. 1. Irrigated and rainfed trial plot, green and yellow circles respectively, yellow cross indicate the tress deprived of irrigation water.

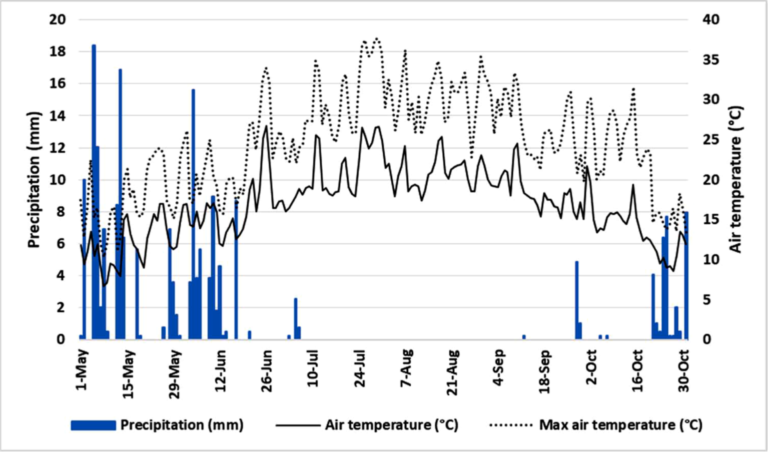

Fig. 2. Daily precipitation (mm), air temperature and maximum air temperature (°C).

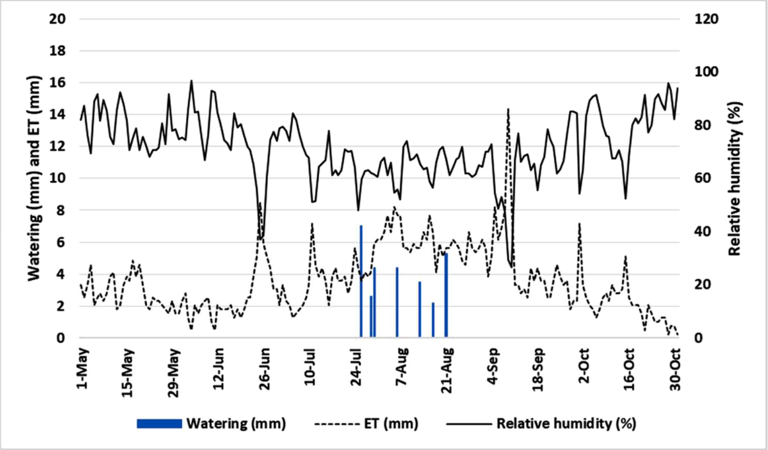

Fig.3. Daily watering (mm), ET (mm) and relative humidity (%).

factorial analysis of variance (ANOVA) with interactions. Factorial design was used to test the following effects: irrigation treatment, canopy aspect, and interactions. Time of day and relevant interactions was also considered for selected variables: stomatal conductance, leaf and stem water potential. Following significant ANOVA, significant differences between treatment levels for different times of day and in the four aspects of canopy leaves were evaluated with Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). Finally, a regression analysis was performed to determine the relationship between midday leaf and midday stem water potential.

4. Results

4.1. *Environmental conditions*

Environmental parameters were collected from May 1 to October 31, and values are shown in Figs. 2 and 3. Precipitation at the study site just before the start of the trial occurred mainly in May and June, amounting to 162.3 mm and 37 mm in September and October (Fig. 2). The average maximum and minimum temperatures were 24.7 ± 6.4 and 10.5 ± 3.5 °C, respectively. Average daily temperatures since June 25 have exceeded 30°C when daily humidity also decreases showing values below 80 %, with an average of 65.3 % from June 25 to August 31 (Fig. 3).

Thereafter irrigation was triggered at times of high evapotranspirative demand and no precipitation when measurements were taken (Fig. 4).

4.2. *Soil water status*

Figs. 4 and 5 show the average soil matric potential values of the five trees at 30, 60 and 90 cm depth. The mean values of soil matric potential decreased rapidly in irrigated trees starting July 26 when irrigation was triggered. At the depth of 30 cm matric potential drops from 160 to 70 kPa, while at 60 cm the response was slower, in fact after about three days a potential of 80 kPa was measured. At the latter depth, the trend in water potential remains consistently higher than at 30 cm. At the depth of 90 cm soil matric potential was almost constant recording average values of 50 kPa (Fig. 4). In rainfed trees, soil was dried down gradually during the experimental period, as shown by the increase in the average values of soil matric potential, considering that the maximum reading of the instrument was 200 kPa. At depths of 30 and 60 cm average values above 190 kPa were reached on August 4, thus leading to possible crop stress conditions. In contrast, at the depth of 90 cm, values of matric potentials stayed lower during the experimental period and did not go beyond about 90 kPa (Fig. 5).

4.3. *Leaf stomatal conductance*

Results of factorial ANOVA for stomatal conductance in the first (26 to 30 July), second (4 to 6 august) and third cycle (14 to 18 august) by treatment, time slot, leaf position in the canopy (aspect) and interactions are reported in Table S3. The time slots refer to four different measurement times, during the day, corresponding to 8–9 a.m. (a), 10–11 a.m. (b), 12–1 p.m. (c) and from 2 p.m. (d). In all measurement cycles, the parameters analyzed, such as treatment, timeslot, and aspect, were significant at $\alpha < 0.05$. Timeslot - aspect interaction is significant in all cycles, while treatment - side interaction is significant in third cycle only.

Mean values of leaf stomatal conductance were 0.28 and 0.35 mol m^{-2} s^{-1} in rainfed and irrigated treatment respectively. There was a gradual divergence between irrigated and rainfed trees in stomatal conductance from the first to the third cycle (Fig. 6). In the first measurement cycle, July 26 to 30, statistically significant differences in average conductance values were recorded in the last day of the cycle only.

In the second and third cycles statistically, significant differences are evident in all days. In the second cycle of measurement, values of 0.25 and 0.32 mol m^{-2} s^{-1} were found in rainfed and irrigated trees respectively, while in third cycle 0.23 and 0.36 mol m^{-2} s^{-1}. Maximum mean values of stomatal conductance ranged between 0.32 mol m^{-2} s^{-1} in rainfed conditions and 0.54 mol m^{-2} s^{-1} in irrigated conditions respectively.

Stomatal conductance takes values above 0.35 mol $m^{-2}s^{-1}$ when VPD is below 2.3 kPa. Above that threshold value, stomatal conductance values decrease over time. VPD and stomatal conductance, are negatively correlated as shown in Fig. 7.

Values from all cycles of measurement were significantly different according to Tukey's test at $\alpha < 0.05$ for irrigation treatments rainfed, time slots (a: 8–9 a.m.; b: 10–11 a.m.; c: 12–13 a.m.; d: from 2 p.m.), and canopy aspect. The interaction, irrigation × aspect, was significantly different only in the third cycle.

Regarding time slots, the mean values of stomatal conductance showed significantly higher values in a and b, followed by c and d of the first and second cycles (Figs. 8b and 9b). In the third cycle, time slot *d* showed significantly lower values than other treatments (Fig. 10b).

In the first cycle mean values showed significantly highest value for the north side, followed by east and south which were not significantly different between each-other. Lowest values were found in the west side. (Fig. 8c); in the second cycle the north and south sides showed higher values than east and west (Fig. 9c) and in the third cycle the north side was significantly highest (Fig. 10c).

The time slot - leaf orientation interaction, in all three cycles, showed in time slot *d* lower mean values of stomatal conductance and statistically significant differences. (Fig. 8d, 9d, 10d). The lowest values of

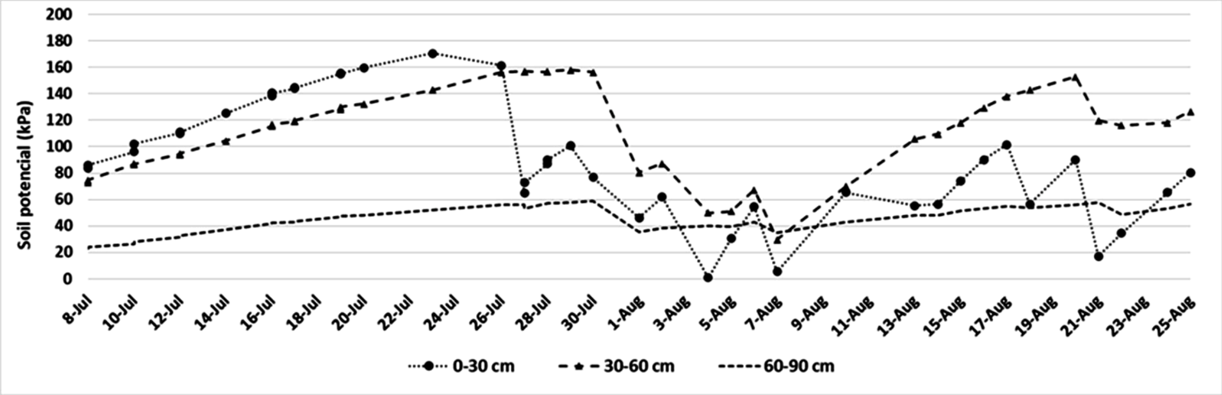

Fig. 4. Average soil matric potential values of the five irrigated trees for each depth.

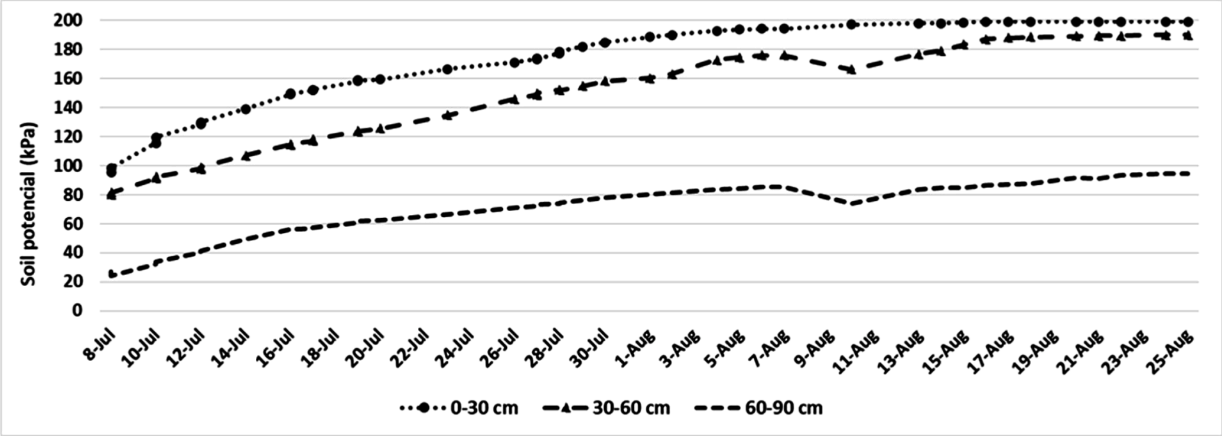

Fig. 5. Average soil matric potential values of the five rainfed trees for each depth.

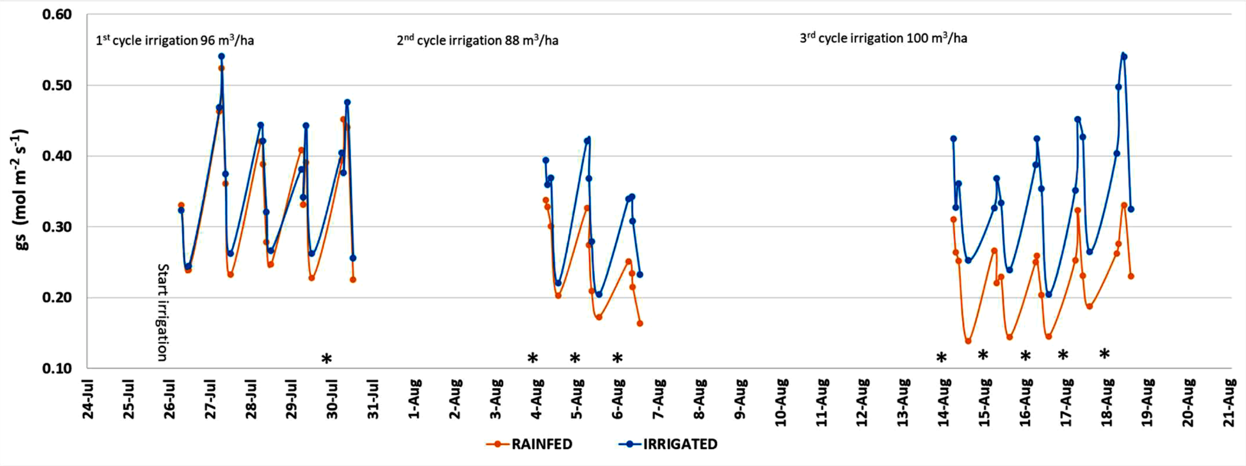

Fig. 6. Stomatal conductance (g_s) trend in rainfed and irrigated trees in first, second and third measurement cycle. Days marked by asterisk differed significantly according to Tukey's test at $\alpha < 0.05$.

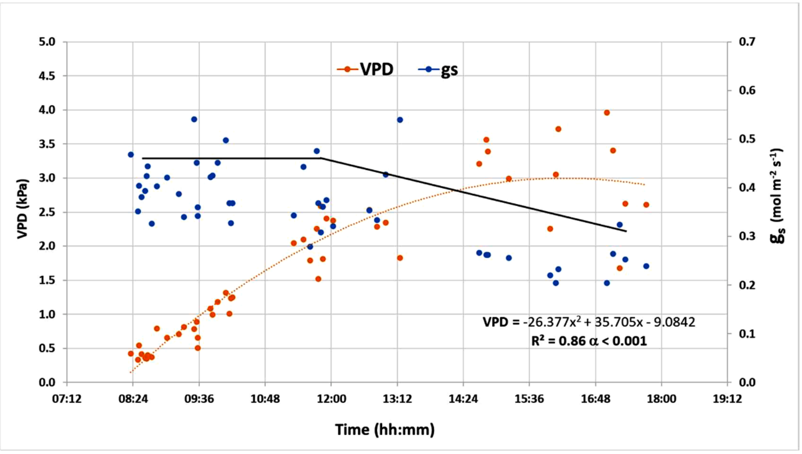

Fig. 7. Relationship between vapor pressure deficit (VPD) and stomatal conductance (g_s) over the diurnal cycle. Solid black line indicates at VPD values of 2.3 kPa the g_s begin to decrease.

stomatal conductance are recorded in the west side of all timeslots and the highest values in the north side. The treatment - leaf orientation interaction showed statistically significant differences in the dry and irrigated treatments of the third cycle (Fig. 10e).

4.4. *Leaf water status: leaf and stem water potential*

Ψ_{PD}, Ψ_{ML} and Ψ_{MS} were measured from July to August 2022 (Table S2). Ψ_{PD} showed relatively constant values in irrigated and rainfed trees (Figure S1). The average values were 0.14 and 0.16 MPa,

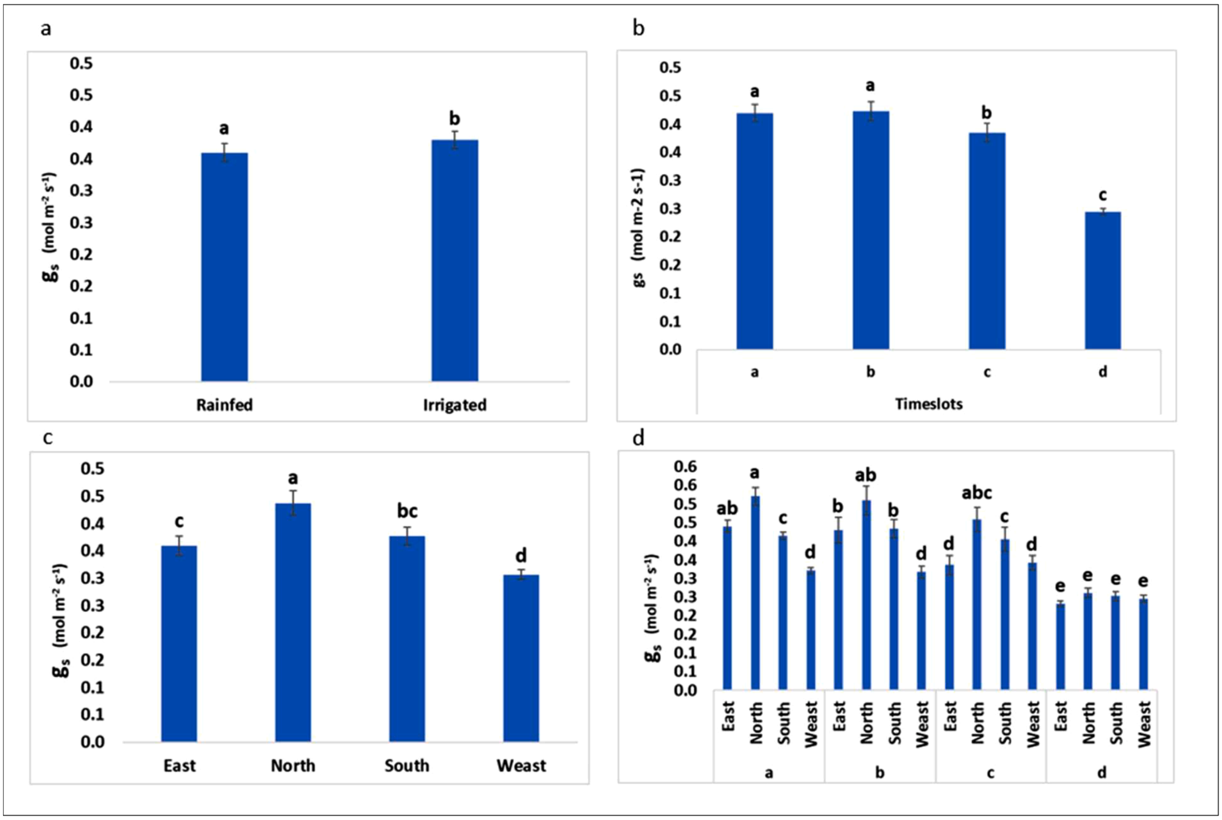

Fig. 8. First cycle: a) leaf stomatal conductance in rainfed and irrigated trees; b) measurements of leaf stomatal conductance in different time slots (a: 8–9 a.m.; b: 10–11 a.m.; c: 12–1 p.m.; d: from 2 p.m.); c) measurements of leaf stomatal conductance in four aspects of leaves canopy; d) interaction timeslots-side. Values with different letters significantly differ according to Tukey's test at α < 0.05 and standard error bars.

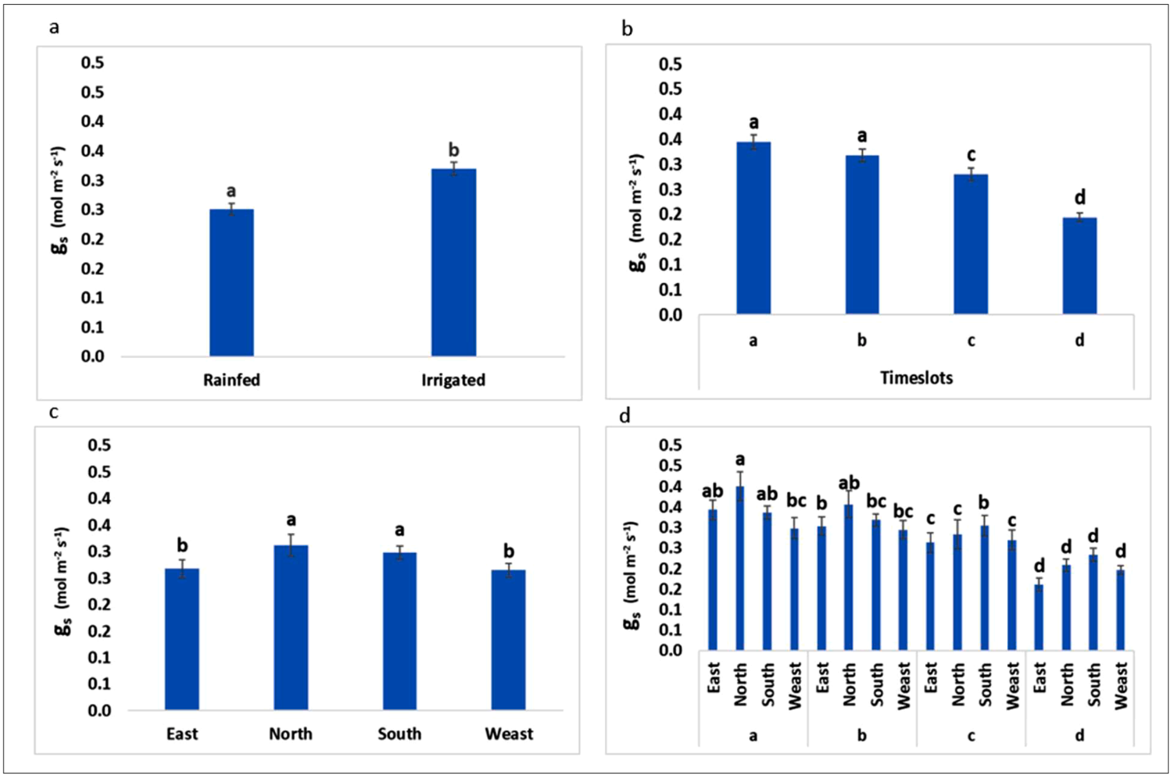

Fig. 9. Second cycle: a) leaf stomatal conductance in rainfed and irrigated trees; b) measurements of leaf stomatal conductance in different time slots (a: 8–9 a.m.; b: 10–11 a.m.; c: 12–1 p.m.; d: from 2 p.m.); c) measurements of leaf stomatal conductance in four aspects of leaves canopy; d) interaction time slots-side. Values with different letters significantly differ according to Tukey's test at α< 0.05 and standard error bars.

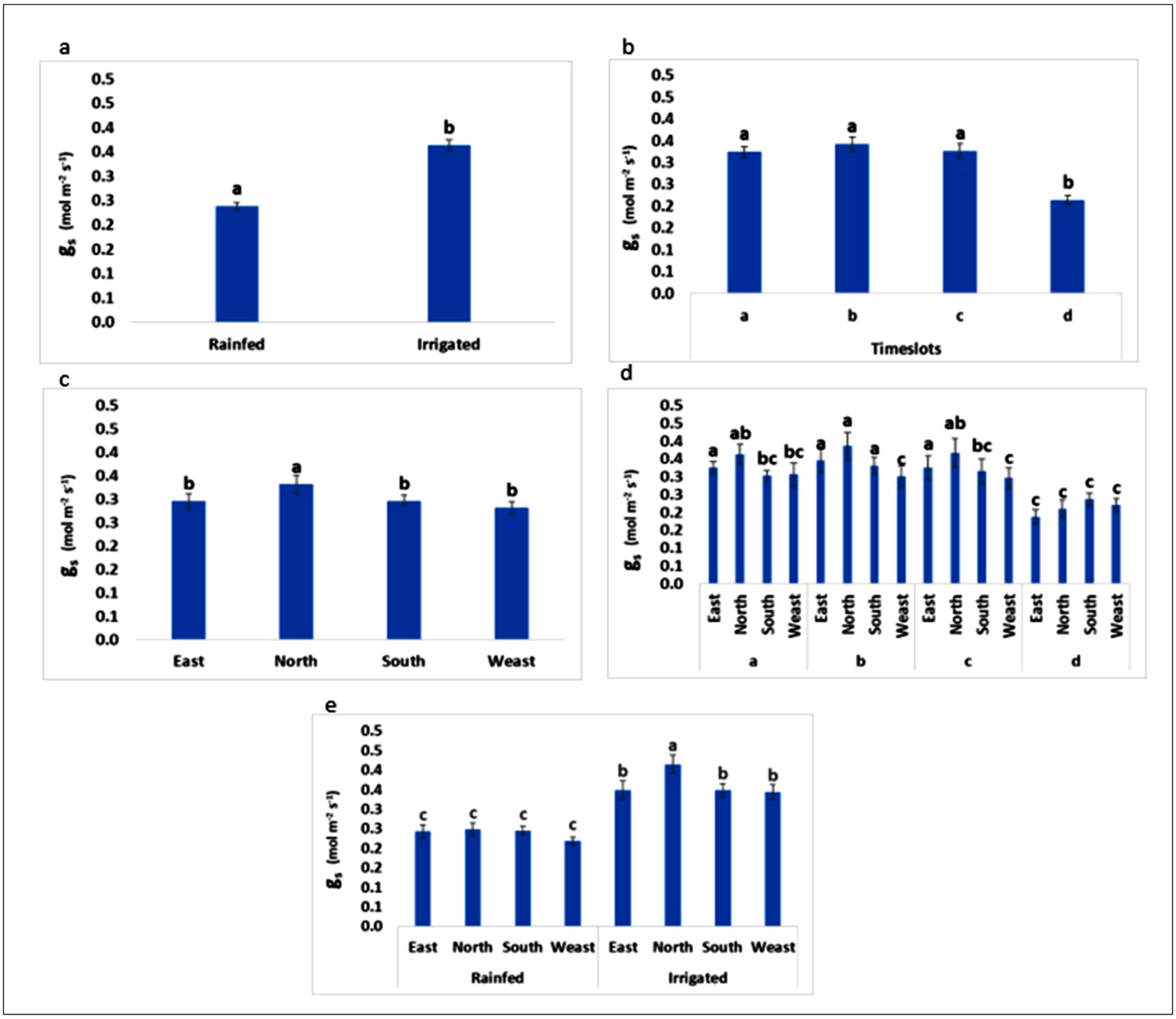

Fig. 10. Third cycle: a) leaf stomatal conductance in rainfed and irrigated trees, with standard error bars; b) measurements of leaf stomatal conductance in different time slots (a: 8–9 a.m.; b: 10–11 a.m.; c: 12–1 p.m.; d: from 2 p.m.); c) measurement of leaf stomatal conductance in four aspects of leaves canopy; d) interaction time slots-side; e) interaction treatment-side. Values with different letters significantly differ according to Tukey's test at $\alpha < 0.05$.

Table 2

Date, average values of ΨPD (MPa), daily maximum temperature, temperature at dew point (°C) and dew hours.

Date	Ψ_{PD} (MPa)	Maximum temperature daily (°C)	Dew point Temperature (°C)	Hourly dew interval	Dew hours
17-Jul	0.22	26.9	11.5	2 a.m. to 6 a.m.	0
19-Jul	0.11	29.8	11.0	3 a.m. to 7 a.m.	4
20-Jul	0.06	35.1	12.5	3 a.m. to 7 a.m.	4
4-Aug	0.09	37.7	10.2	1 a.m. to 6 a.m.	6
5-Aug	0.14	33.5	9.1	–	0
6-Aug	0.13	36.6	10.0	3 a.m. to 7 a.m.	4
21-Aug	0.22	33.8	12.2	12 a.m. to 8 a.m.	8
24-Aug	0.11	36.6	12.8	12 a.m. to 8 a.m.	8

respectively. Table 2 shows the average values of Ψ_{PD} per each day of measurements with dew temperature and dew persistence time on leaves. Ψ_{ML}, and Ψ_{MS} instead, showed a slight increase over time. The ΨML showed average values of 1.27 MPa in rainfed trees and 1.30 MPa in irrigated trees, while the same mean value of 1,00 MPa was obtained for Ψ_{MS} in both treatments. Ψ_{ML} values were always higher than the Ψ_{MS}.

The regression equation in Fig. 11, between ΨML and ΨMS showed a positive relationship

(R^2 0.75; α=0.0000). The results indicate that the two methods are linearly and positively related to each other.

The ANOVA results for Ψ_{PD}, Ψ_{ML} and Ψ_{MS} in the measurements of the first (July 26–30), second (August 4–6) and third cycle (August 14–18) of treatment (irrigated and rainfed trees), leaf orientation on the canopy (side) are shown in Table S4. The effects of all factors tested, treatment, side and interactions assumed different behavior compared to results obtained for stomatal conductance.

In the first cycle of measurements, no statistically significant differences were found in any of the factors tested. Statistically significant differences occurred in the second and third cycle. Significance statistically differences occurred in the treatment in second cycle of Ψ_{ML} and in third cycle of Ψ_{MS} and $\Psi_{PD.}$ Differences between aspects were found in the second and third cycle of all potentials.

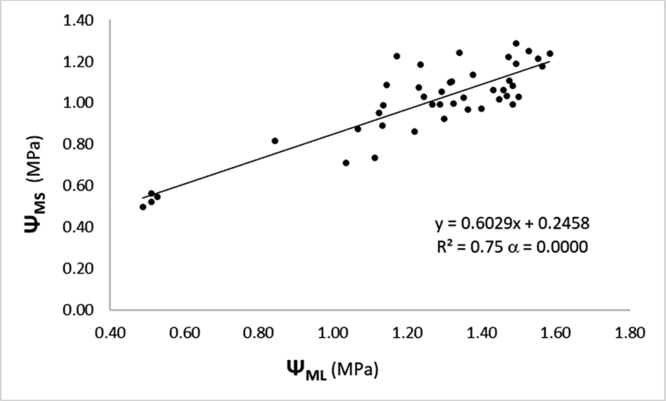

Fig. 11. Relationship between Ψ_{MS} (midday stem) and Ψ_{ML} (midday leaf) in 'McDonald' hazelnut. Each point is the average of four leaves in the canopy.

In Ψ_{ML} rainfed and irrigated trees showed a value of 1.31 and 1.40 MPa respectively (Fig. 12a). The Ψ_{ML} values, in second cycle for the side were ranked as follows: South (1.49 MPa) > East (1.39 MPa) > West (1.32 MPa) > North (1.23 MPa) (Fig. 12b). Instead, in third cycle south (1.53 MPa) and west side (1.52 MPa) showed highest mean values than east (1.39 MPa) and north (1.36 MPa) side (Fig. 12c).

The Ψ_{MS} mean values, in rainfed and irrigated trees, in the third cycle, were 1.12 and 1.02 MPa respectively (Fig. 13a). West (1.13 MPa) and south (1.10 MPa) values were highest and statistically different, than east (1.01 MPa) and north (1.01 MPa) side, in second cycle (Fig. 13b). In the third cycle west side leaves showed a value of 1.15 MPa which was significantly different from all other orientations (Fig. 13c).

In the third cycle of Ψ_{PD} mean value were significantly higher in irrigated rainfed trees (Fig. 14a). Leaves orientation, in second cycle, were ranked as follows: South (0.15 MPa) > West (0.14 MPa) > North > (0.13 MPa) > Est (0.11 MPa) (Fig. 14b). In the third cycle the North side showed a value of 0.06 MPa which was significantly lowest than all other orientations (Fig. 14c)

5. Discussion

5.1. Stomatal conductance

Stomatal conductance, in hazelnut trees, responds rapidly to induced stress, even if mild, and can be considered an indicator for detecting early signs of stress. The differences between rainfed and irrigated trees were already evident in the first cycle of measurements, four days after irrigation started. As irrigation continued, the differentiations between the two treatments became more evident. In addition, from a methodological point of view, the study of measurements taken on the four sides of canopy aspects and the timeslots are highly relevant. The north and west canopy aspects were detected for the first time, with higher and lower values, respectively, that best express the plant's physiological response in terms of stomatal conductance.

The highest values of stomatal conductance were reached in the morning until 11 a.m. and from midday, the values decreased, in agreement with the findings of Pasqualotto et al., 2018 and Özmen (2016). The average stomatal conductance decreased when VPD values reached 2–2.3 kPa from midday, while Pasqualotto et al., 2018 reported values of 1.5 kPa. Thereafter, the decrease in transpiration is driven by the decrease in VPD in accordance with findings in hazelnut (Girona et al., 1994; Tombesi, 1994; Hogg et al., 2000; Pasqualotto et al., 2018) and in other species such as olive tree (Moriana et al., 2002) pine, (Awada et al., 2002) and rose (Samartzidis et al., 2005).

5.2. Leaf and stem water potential

Predawn leaf water potential, leaf water potential at midday, and stem water potential at midday can be used as indicators for the plant and soil water status (Koide et al., 1989; Amèglio et al., 1994 ; Naor,

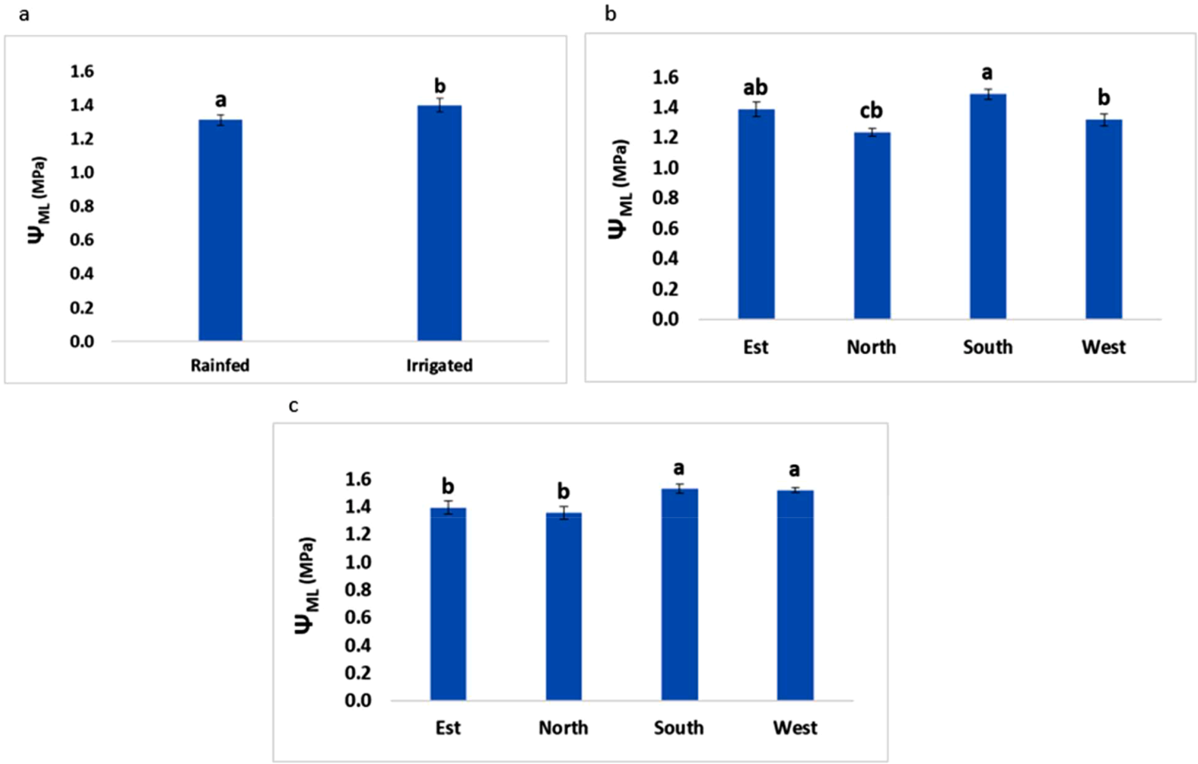

Fig. 12. Ψ_{ML}: a) rainfed and irrigated trees in second cycle,; b) leaves canopy aspects in second cycle; c) leaves canopy aspects in third cycle in rainfed and irrigated trees, with standard error bars Values with different letters significantly differ using Tukey's test at $\alpha < 0.05$.

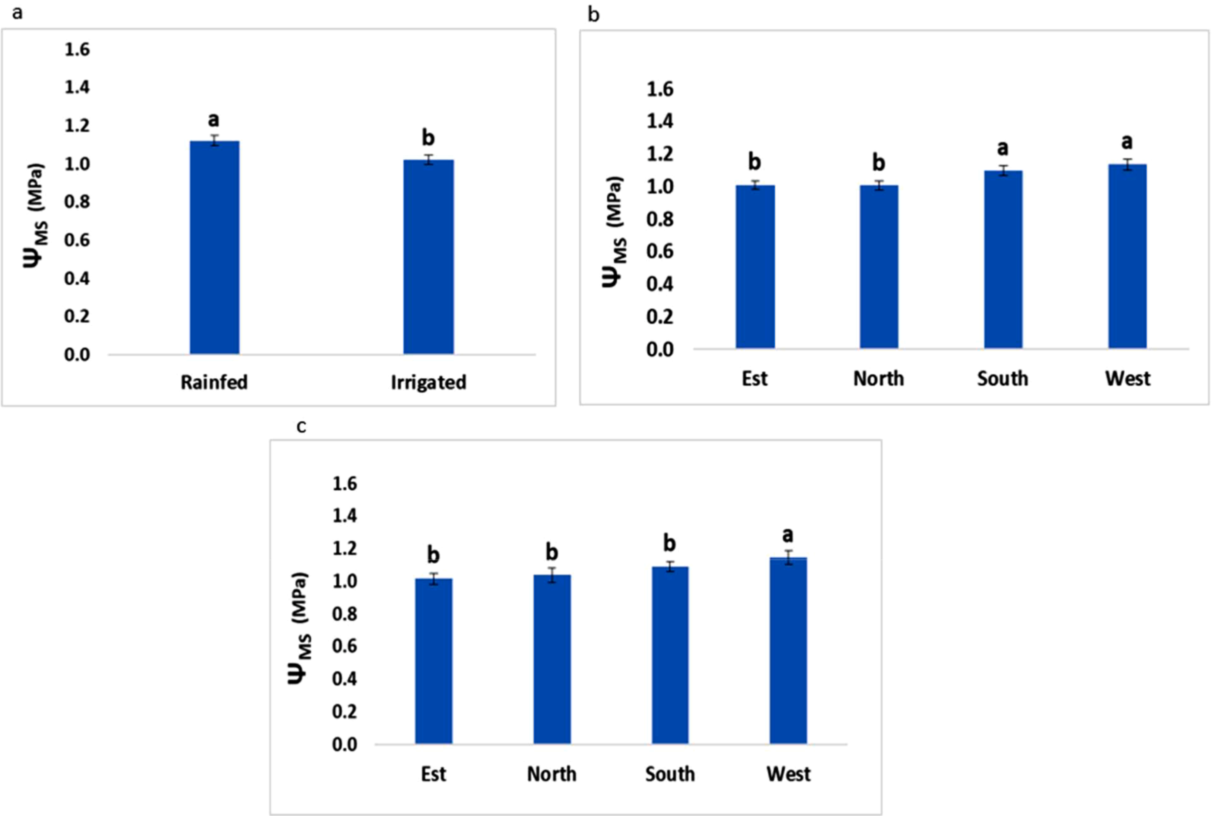

Fig. 13. Ψ_{MS} in: a) rainfed and irrigated trees in third cycle; b) Aspects of leaf of the canopy in second cycle and c) third cycle. Values with different letters significantly differ using Tukey's test at $\alpha < 0.05$ and standard error bars.

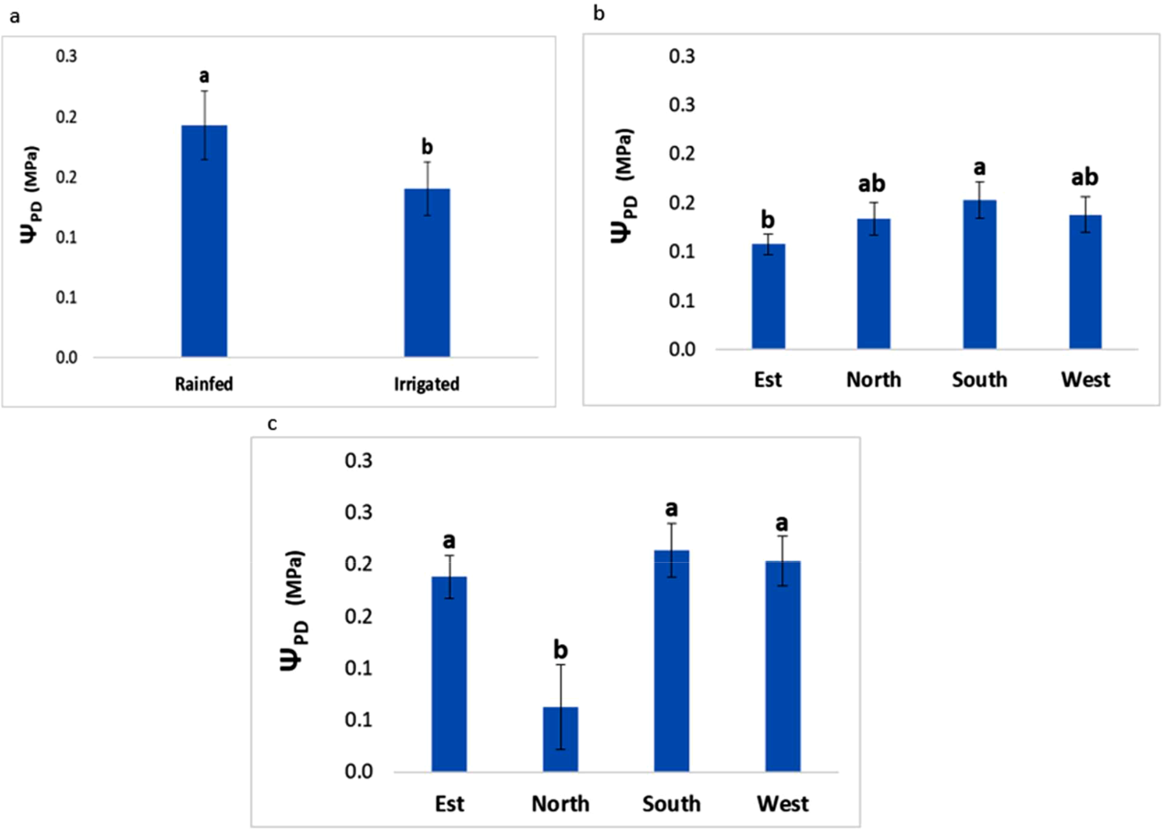

Fig. 14. Ψ_{PD} in: a) rainfed and irrigated trees in third cycle b) Aspects of leaf of the canopy in second cycle and c) third cycle. Values with different letters significantly differ using Tukey's test at $\alpha < 0.05$ and standard error bars.

2000; Williams and Araujo, 2002 ; Chastain et al., 2014; Knipfer et al., 2020). In the analysis by factorial ANOVA, potentials do not show statistically significant differences in all cycles. This explains that differences between treatments in water potentials could occur with more severe water stress conditions. Besides, hazelnut needs about 800 mm of precipitation distributed throughout the year (Cristofori et al., 2014) and, in this study 20% of the rainfall fell down in May and June contributing to soil moisture reserve as also observed by Özmen (2016). In fact, soil matric potential soil didn't reach a high value in rainfed trees at maximum survey depth. This indicates that the triggering of irrigation did not have much influence on soil matric potential at this depth.

Ortega-Farias et al. (2020) found no significant differences, particularly for stem water potential, at the beginning of the water restriction period, but differences became clear about a week later. However, water potential does not depend only on soil water content but is affected by the environment in which the plants are located and their interactions with the environment (García-Tejera et al., 2021). In our study, drought stress may not have been severe enough to show clear differences between water potential in irrigated compared to rainfed trees.

The evaporative demand of the environment and the transpirative activity of leaves have resulted in Ψ_{ML} measurements higher than Ψ_{MS} to maintain the potential gradient of the soil-plant-atmosphere system. In fact, Ψ_{ML} reached average values of 1.3 MPa versus 1.0 MPa of the Ψ_{MS}. Measured values of Ψ_{ML} showed a general enhancement during the measured period in accordance to Özmen (2016) in rainfed hazelnut trees. Ψ_{ML} and Ψ_{MS}, show a strong correlation with each other, which is the reason why they could be used indiscriminately to define hazelnut water stress reference values, as also demonstrated by Williams and Araujo (2002) in grapevine. However, considering that the hazelnut is very sensitive to the evaporative demand of the environment, independently of soil water availability (Pasqualotto et al., 2018), it is preferable to use Ψ_{MS} which it less variable than Ψ_{ML} to environmental conditions, as highlighted by McCutchan and Shackel (1992) and Naor (2000) in fruit trees. The average values of Ψ_{PD} are very low in rainfed and irrigated trees, 0.16 and 0.14 MPa, respectively. Data from the literature suggest Ψ_{PD} values between 0.1 and 0.42 MPa (Marsal et al., 1997; Girona et al., 1994) 0.4 and 0.9 MPa (Awada, 2007). The low Ψ_{PD} values found in this study were associated to the frequent reaching of the dew point, which led to water condensation on the leaves. In fact, our study, on days of Ψ_{PD} measurement, were reached lasting conditions of presence of dew, probably responsible of leaf water recovery. Burgess and Dawson (2004) state that leaf wetting events, especially in plants with weak stomatal control, could help restore the amounts of water lost in the previous day and thus suppress leaf water loss. Occult water, absorbed by leaves, may play an important role in plant physiology (McHugh et al., 2015; Tomaszkiewicz et al., 2015). Fog water absorbed by leaves and transported to the xylem improved leaf water potential, photosynthesis, stomatal conductance and growth compared to plants unexposed to fog water (Eller et al., 2003). This indicates that occult moisture, typical of specific environments, such as Willamette Valley, interacts with plant physiology and this would allow plants such as hazelnut, originally an understory plant, to take advantage of such events in overcoming heat and drought stress, especially in the early part of the day. This phenomenon should be investigated in hazel because although there is much work supporting uptake through the leaf surface of occult moisture and mist water, it does not represent the main pathway for leaf water uptake among all species (Berry et al., 2019).

Soil water potential and leaf water potential could not be related because of the limitation of the instrument's measurement, which was up to 200 kPa. Although the induced water stress was mild during the experimental period, the increase in water potential over time suggests that, under those environmental conditions, it is due to the evaporative demand of the environment independent of soil water availability.

6. Conclusions

The application of water stress, although mild, resulted in large differences in hazelnut trees physiological behavior. Water potential and stomatal conductance showed different sensitivity in establishing the response to stress of trees under irrigated and non-irrigated condition.

Leaf conductance measurements were found to be useful, easy to interpret and extremely sensitive, compared to leaf and stem potential measurements, because of the ability of the hazelnut trees to regulate stomatal opening under mild stress conditions. Hence, this study indicates leaf conductance as a potential early indicator of stress. Besides, data show that stomatal conductance should be measured in the north and west aspects which assume the highest and lowest of the range, respectively. Time of day also plays a key role in the measurement of plant stress. Measurements from 11 a.m.- 12 p.m. are more appropriate for detecting stress conditions.

Unlike stomatal conductance, however, water potential does not show a rapid response to stress, so it may not be considered in this study as a method to detect early signs of stress. Results show that there are no statistically significant differences, between the mean values of Ψ_{ML}, Ψ_{MS} and Ψ_{PD} in irrigated and rainfed trees, in the second and third measurement cycles. No differences were found between the different canopy leaves orientations on which measurements were taken. However, the northeast and southwest sides showed similar behaviors, with lower and higher mean values, respectively.

Based on the results, we suggest that further research is needed on the usefulness of water potentials as early indicators of water stress in hazelnut, since in our data differences between irrigated and rainfed treatments only occur when the stress becomes significant. Furthermore, the high positive correlation between leaf and stem water potential connotes them as equivalent methods for measuring plant water status.

From a methodological point of view this research fills a gap in knowledge relative to the hazelnut about the variability in potential and stomatal conductance response related to canopy aspect. Leaf stomatal conductance showed different responses in relation to leaf location on the canopy, and the North and West positions showed to represent the extreme values in all measurement cycles. This may guide measurements on trees if the focus is investigating areas with major differences in physiological responses.

Further studies on the role of hidden precipitation water absorbed by leaves in hazelnut, especially in areas with large temperature excursion, would be useful.

CRediT authorship contribution statement

Gessica Altieri: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft. **Nik G Wiman:** Methodology, Writing – review & editing. **Francesca Santoro:** Formal analysis, Software, Writing – review & editing. **Mariana Amato:** Resources, Supervision, Visualization, Writing – review & editing. **Giuseppe Celano:** Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112817.

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