

Irrigation improves tree physiological performances and nut quality in sweet chestnut

Giulio Demetrio Perulli^{*}, Alexandra Boini, Brunella Morandi, Luca Corelli Grappadelli and Luigi Manfrini

Distal - Department of Agricultural and Food Sciences, University of Bologna, V. le Fanin 46, 40127 Bologna, Italy

*Corresponding author: <u>giulio.perulli@unibo.it</u>; Tel.: +39 339 2527728

Received: 17 December 2021; Accepted 12 April 2022; Published: 29 April 2022

Abstract: Italy is one of the most important world chestnut producers. The majority of traditional sweet chestnut orchards are still non-irrigated since they are typically located in mountain-hill areas usually characterized by environmental conditions that are not limiting for the vegetative and reproductive growth of this fruit tree crop. Nowadays, the increase of summer temperatures and the decrease of rainfall are affecting negatively chestnut physiological performances and productivity. The adoption of scheduled irrigation practices, in light also of the limited water availability/possibility of storage (e.g., artificial lakes, reservoirs) of these areas, should become part of chestnut orchard management. The aim of the present study was to evaluate the effect of irrigation on sweet chestnut physiology, nut quality and yield. The study was carried out in 2020 in a traditional chestnut orchard of the "Marron Buono di Marradi" ecotype, located in the Tuscan-Emilian Apennines (Marradi, Italy). The experimental design compared trees irrigated between August and September with a non-irrigated control. Leaf gas exchange and plant water status were monitored during the growing season and, nut quality and yield were assessed at harvest. Results showed that irrigated trees exhibited, in middle September, higher photosynthesis, transpiration, stomatal conductance and stem water potentials compared to the non-irrigated control trees. Nut size was significantly smaller in non-irrigated trees than in irrigated ones while the yield was not statistically affected by the irrigation treatment. Despite the favourable mild and rainy weather conditions occurred in 2020, the application of irrigation during the nut filling phase (e.g., late summer) was beneficial for enhancing sweet chestnut physiological performances and for improving nut quality.

Keywords: Castanea sativa Mill., chestnut physiology, leaf gas exchanges, water relations, nut yield

1. Introduction

Nowadays climate change is causing more frequent and prolonged drought periods also in mountain-hills areas where water was not a limiting factor for chestnut (*Castanea sativa* Mill.) cultivations in the past century. The overall increase of summer temperatures, the decrease in total precipitation amount, and the change in their distribution during the year are having a negative impact on the productivity performance of traditional rainfed chestnut cultivations (Perulli et al., 2020). The lack of rain at the end of summer and/or in autumn is known to be the main constrain for chestnut development and production, bringing income losses to the chestnut sector (Vida Rural, 2017). Rainfall in August and September is known to be positively correlated to chestnut tree productivity (Vigiani, 1941; Breisch et al., 1995; Bounous, 2002; Gomes-Laranjo et al., 2007; Vida Rural, 2017; Mota et al., 2018b). Furthermore, recent studies reported that also burr absolute growth rate and burr size at harvest were positively influenced by total precipitations, especially by those occurring in August and September (Perulli et al., 2020).

The introduction of irrigation as a common orchard management practice could be the solution for

both promoting the resilience of chestnut orchards to climate change and to allow more regular and greater production with higher nut quality (e.g., size) over the years. Unfortunately, in chestnut orchards, irrigation is still barely adopted worldwide, due to many difficulties like the shortages of water availability (e.g., artificial lakes or water reservoirs) for irrigation purposes in the typical chestnut cultivation areas (i.e., mountain-hills areas). This is especially true in Italy, even though the Italian Apennines (Mount Amiata, Tuscany) are among the first documented areas where chestnut irrigation was firstly adopted at the beginning of XX century (Vigiani, 1908). Furthermore, in most growing countries (e.g., Italy), chestnut is still managed following mainly traditional practices and the number of orchard plantations cultivated as "specialized orchards" following the fundamentals of the modern horticulture, is still scarce. To date, only some countries, like Portugal, lately started to study and to apply irrigation practices in specialized chestnut orchards (Vernol, 2013). These studies mainly aimed to evaluate chestnut water management based on plant physiological performances (e.g., leaf gas exchange and plant water relations) and to evaluate the adoption of different irrigation systems to set out the best irrigation method for this tree crop (Mota, et al., 2017). Linhares et al. (2005) studied the effect of irrigation, likely managed on farmer knowledge, on nut production and quality in a chestnut orchard located in Northeast Portugal. The more recent studies, instead, tried to set chestnut water requirements based on predawn leaf water potential thresholds (e.g., Ψ_{pd} : -0.6 MPa) and balancing the reference evapotranspiration for the experimental area conditions (Martins, et al., 2010, 2011).

Mota et al. (2014) considered Ψ_{pd} and midday leaf water potential (Ψ_{md}) for setting up full and deficit irrigation treatments. Recently, Mota et al. (2018c) applied the threshold of -1.2 MPa on the midday stem water potential to trigger the irrigation. Most of these studies highlighted that irrigation improves tree physiological performances, in terms of photosynthetic rate, transpiration rates and water potential compared to non-irrigated treatments (Mota et al., 2014, 2018b; Gomes-Laranjo, et al., 2018). Irrigated trees also showed higher yields and bigger nut size without affecting their chemical and sensorial composition (Mota, et al., 2018c). Therefore, irrigation is considered to be a suitable strategy to improve chestnut productivity and quality in terms of nut size, but also it could allow more regular and greater production over the years (Gomes-Laranjo et al., 2018; Perulli et al., 2020). However, to date, studies related to an irrigation management applied at specific phenological stages (e.g., nut filling) are limited even if it is widely known that nut growth and production are positively affected by precipitations occurring at the end of summer/beginning of autumn (Mota et al., 2018a). In *C. mollissima* Blume, this seems to coincide with the increase of nut endosperm dry mass (i.e., starch accumulation) (Chen et al., 2017). Optimal soil humidity condition achieved at this time would likely enhance plant physiological performances (e.g., carbohydrate synthesis) and thus tree productivity.

The application of irrigation practices on chestnut orchard at specific phenological stages (i.e., nut filling) could be among the best applicable management strategies for increasing plant productivity and at the same time saving water in areas characterized by limited availability of water resources. Indeed, the differential phenological sensitivity of fruit yield and composition to irrigation was demonstrated in many important fruit tree crops as peach, grapevine and kiwifruit (Naor et al., 2006; Basile et al., 2012; Torres-Ruiz et al., 2016).

The aim of this research was to evaluate the effect of irrigation applied during the nut filling phase (e.g., end of summer) on physiological and yield performances of a traditional sweet chestnut (*C. sativa* "Marron Buono di Marradi") orchard located in the Italian Apennines.

2. Material and Methods

2.1. Orchard location and weather conditions

The study was carried out in 2020 in Albero locality, Marradi (Firenze, Italy), located in the Tuscan-Emilian Apennines (44°02' N, 11°36' E) at 550 m elevation, in a commercial (about 35 years old), rainfed, sweet chestnut orchard (*Castanea sativa* Mill.). Trees were of the "Marron Buono di

Marradi" ecotype ('Marroni' type) grafted on seedling rootstocks. This ecotype is characterized by elevated vigour, expanded vegetative habit and high productivity (Fideghelli, 2016). The orchard is located close to a traditional centuries-old chestnut orchard, in a nearly flat area due to an ancient landslide. The soil is mostly classified as deep Haplic cambisols (Dystric), with a sandy loam texture. For the present study, dates were shown in DAFB (days after full bloom), with full bloom that occurred on June 25th. DAFB were used to keep the same reference time of a previous study on *C. sativa* carried out in the Tuscan-Emilian Apennines (Perulli et al., 2020).

Weather conditions were measured *in-situ* by a weather station (Winet srl, Cesena, Italy) recording mean and maximum air temperatures (°C), air relative humidity (%) and precipitation (mm) from 44 to 116 DAFB. From November 2019 to July 2020, rainfall was measured by a weather station located in Marradi belonging to the Regional Agency for Environmental Control (Arpae, 2021), whereas from August to October 2020 this parameter was measured by the *in-situ* weather station (Winet srl, Cesena, Italy).

The climate of Marradi area was classified, according to Köppen e Geiger classification, as Cfb (Temperate oceanic climate) with the data provided by ECMWF (European Centre for Medium-Range Weather Forecasts) on weather data (e.g., precipitation) collected in the 1999-2019 historical period (Climate-Data, 2021).

2.2. Experimental set-up

The study was set-up with two treatments: irrigated (I) and non-irrigated (NI) trees. Two blocks, with three trees per block, were established for each treatment. Irrigation was performed at 58, 80 and 87 DAFB, supplying manually (with an irrigation hose), 10 mm of water under the canopy projection, corresponding to 270 L tree⁻¹. Water was taken by a close natural spring, collected in plastic tanks and then electrically pumped to the orchard for the irrigation supply. The dates and volumes of irrigation were selected taking into account the nut filling phenological stage, the spring water availability and the rainfall and temperature trend during the considered period.

2.3. Physiological performances

2.3.1. Leaf gas exchanges

Leaf photosynthesis (A), transpiration rate (E) and stomatal conductance (g_s) were measured at midday on the following two dates: 64 (28 August) and 82 DAFB (15 September). These measurements were carried out using an open-circuit infrared gas exchange analyzer fitted with a LED light source (Li-COR 6400, LI-COR, Lincoln, NE, USA) on two well-exposed leaves per tree, on 6 trees per treatment. During each measurement, light intensity was maintained constant, setting the LED light source to the natural irradiance (which was always above 1,700 µmol m⁻² s⁻¹) experienced by the leaves immediately before the measurement, while air CO₂ concentration was set at 400 ppm.

2.3.2. Water relations

Stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials were also measured at midday, 64 and 82 DAFB, using a Scholander pressure chamber (Soilmoisture Equipment Corp., Goleta, Santa Barbara, CA, USA). To measure leaf water potential, two well-exposed leaves per tree, on 6 trees per treatment, were chosen and analyzed right after excision, following the protocol of Turner and Long (1980). On the same trees where leaf water potentials were measured, two leaves per tree located in the inner part of the canopy, as close as possible to the trunk, were chosen to measure stem water potential. The selected leaves were covered with an aluminum foil and enclosed in a plastic bag for at least 90 minutes before the measurement to reach equilibrium. Then, water potential was measured, just after excision, according to the methodology reported by Naor et al. (1995).

2.3.3. Tree nut quality and yield

Harvest was performed manually under each tree in two times, on 19 and 28 October (116 and 125 DAFB, respectively), due to the progressive nut fall. The nearly flat area of the orchard facilitated to easily distinguish and to harvest the nuts from each single tree with the help of a rake and a blower.

Then, nuts from each tree were weighted to calculate the tree yield (kg tree⁻¹). All the nuts were included in the calculation of the yield. Nut quality was assessed, excluding the nuts aborted, rotten, and with the worm presence (e.g., *Cydia splendana*), on 30 nuts per tree (180 nuts per treatment), by measuring nut fresh weight (g fruit⁻¹) and nut maximum diameter (mm) with a precision scale (Model PE3600, Mettler Toledo LLC, USA) and with a caliber provided with an external memory (Calibit, Mitutoyo Absolute Digimatic series 500), respectively. From the values of nuts weight, it was indirectly calculated the number of nuts per kg, that is the conventional way to express nut quality for the chestnut market.

Based on the number of nuts per kg, a nut class size distribution was calculated following the Italian market indication for the fresh product (Decreto Ministeriale,1939): below 50 nuts kg⁻¹ size classes were set every two nuts per kg difference (e.g., 38/40 nuts kg⁻¹), while between 50 nuts and 100 nuts kg⁻¹, size classes were set every five nuts per kg difference (e.g., 70/75 nuts kg⁻¹).

Nut fresh weight (FW) was determined with a high precision balance (Model PE3600, METTLER TOLEDO LLC, USA). Once weighted for the FW, nuts were put in an oven at 65 °C for seven days and weighted again to get the dry weight (DW). Dry matter percentage (%DM) was calculated with the following formula:

$$\%DM = \frac{DW}{FW} \times 100$$

2.4. Statistical analysis

Leaf gas exchange, water relations, nut quality and tree yield data were compared between the treatments using a one-way ANOVA analysis. Analyses were carried out using R software (www.rproject.org).

3. Results

3.1. Weather conditions

The annual precipitations registered in 2020 if compared to the average of historical precipitation data collected in 1999-2019 period in the same area (Climate-Data, 2021), were 1232 and 898 mm, respectively (Table 1) while the cumulative precipitations of November-March in 2020 and in 1999-2019 period were 736 and 414 mm, respectively. As concerning the burr growth development period (July-October), the total rainfalls were 359 and 252 mm in 2020 and in 1999-2019, respectively. August was the summer month with the highest cumulated precipitations recorded (79.4 mm; Table 1). An intense rainfall of 38.6 mm was registered on 16 August (52 DAFB) (Figure 1).

Precipitations started to occur more frequently but with a lower intensity on 21 September (88 DAFB; Figure 1). Mean daily temperatures (T_{med}) were overall mild, with 22 °C and 17.6 °C registered in August and September, respectively. A T_{max} above 32 °C was registered in 4 days (47, 48, 57, 59 DAFB) along the season (Figure 1). T_{max} started to sharply decrease on 21 September (88 DAFB). The mean air relative humidity and VPD were 70% and 1.8 kPa in August and 78% and 1.6 kPa in September, respectively.

| Season | Rainfall (mm) | | | | | | | |
|------------|---------------|---------|---------|------|------|------|------|--|
| | Annual | Nov-Mar | Apr-Jun | Jul | Aug | Sep | Oct | |
| 2020 | 1,232 | 736 | 167 | 73.2 | 79.4 | 69.7 | 127 | |
| 1999-2019* | 898 | 414 | 232 | 44.0 | 41.0 | 72.0 | 95.0 | |

Table 1. Precipitations registered in Marradi area (FI) in 2020 and during 1999-2019 historical period.

*Season refers to average values of historical data collected in 1999-2019 period in Marradi area. Data were provided by Climate-Data (2021).



Figure 1. Weather conditions from 40 DAFB (4 August 2020) to 114 DAFB (19 October 2020).

3.2. Leaf gas exchanges

The photosynthetic rate (A), measured at 64 DAFB, did not show statistical differences between treatments (11.7 and 14.4 mmol CO₂ m⁻² s⁻¹ for irrigated (I) and non-irrigated (NI) trees, respectively) (Figure 2). Similarly, the transpiration rate (E) at 64 DAFB was not statistically different between the treatments with values of 3.4 and 3.7 mmol H₂O m⁻² s⁻¹, respectively for I and NI trees (Figure 2). The stomatal conductance (g_s) at 64 DAFB also did not show statistical differences between the treatments with values of 0.13 mol H₂O m⁻² s⁻¹ and 0.14 mol H₂O m⁻² s⁻¹, respectively for I and NI treatments (Figure 2).

At 82 DAFB, A was significantly higher in I than NI trees, with values of 19.6 and 15.1 mmol CO_2 m⁻² s⁻¹, respectively (Figure 2). E was significantly higher for I (3.7 mmol H₂O m⁻² s⁻¹) than for NI (2.8 mmol H₂O m⁻² s⁻¹) trees at 82 DAFB (Figure 2). Similar results were measured for g that was significantly higher in I compared to NI trees, with values of 0.17 and 0.13 mol H₂O m⁻² s⁻¹, respectively.



Figure 2. Leaf gas exchange parameters measured at 64 and 82 DAFB for the irrigated (I; light blue columns) and non-irrigated (NI; ocher columns) treatments (mean \pm SE). *: effect significant at P \leq 0.05. Recorded air temperatures (°C) at 64 and 82 DAFB were 32.2 \pm 0.17 and 27.8 \pm 0.14, respectively.

3.3. Water potentials

Midday stem (Ψ_{stem}) and leaf water potentials (Ψ_{leaf}) measured at 64 DAFB did not show statistical differences between the treatments, with mean Ψ_{stem} values of -0.92 and -0.95 MPa and mean Ψ_{leaf} values of -1.68 and -1.69 MPa, respectively for I and NI trees (Figure 3).

At 82 DAFB, Ψ_{stem} of I trees was significantly higher (-0.90 MPa) than in NI trees (-1.01 MPa) (Figure 3). No differences were instead recorded for Ψ_{leaf} , with -1.55 MPa and -1.58 MPa registered for I and NI trees, respectively (Figure 3).



Figure 3. Midday stem and leaf water potentials recorded at 64 and 82 DAFB for the irrigated (I; light blue columns) and non-irrigated (NI; ocher columns) treatments (mean \pm SE). *: effect significant at $P \le 0.05$

3.4. Nut quality and tree yield

As concern nut quality, I trees showed statistically higher values compared to NI for the following parameters: nut fresh weight (14.9 and 14.2 g nut⁻¹), number of nuts per kilogram (68.8 and 73.5 nuts kg⁻¹), nut diameter (37.6 and 36.9 mm), respectively for I and NI treatments (Table 2).

Nut dry matter was similar between treatments with values of 46.9 and 47.1% for I and NI trees, respectively. The nut yield per tree was not significantly different between the treatments with values of 43.3 kg tree⁻¹ and 37.4 kg tree⁻¹ for I and NI, respectively (Table 2).

Table 2. Nut quality parameters and nut yield per tree for irrigated and non-irrigated treatments (mean±SE).

| Treatment | Nut fresh weight | Nut maximum diameter | Nut size | Nut dry matter | Nut yield |
|--------------------|---------------------------|-------------------------|-------------------------|-------------------|--------------------------|
| | (g nut ⁻¹) | (mm) | (nut kg ⁻¹) | (%) | (kg tree ⁻¹) |
| Irrigated (I) | $14.9 \pm 0.17 \text{ a}$ | 37.6 ± 0.18 a | $68.8\pm0.82~b$ | 46.9 ± 0.17 | 43.3 ± 2.89 |
| Non-irrigated (NI) | $14.1\pm0.23~b$ | $36.9\pm0.21\ b$ | 73.5 ± 1.22 a | 47.0 ± 0.22 | 37.3 ± 5.64 |
| Significance | * | * | * | ns | ns |

Means followed by different letters are significant at $P \le 0.05$ (*). ns: effect not significant.



Figure 4. Nut size class distribution for irrigated (I; light blue columns) and non-irrigated (NI; ocher columns) treatments.

The frequency distribution of the nut into size classes indicated that the most represented classes, for both the treatments, were 60/65, 65/70 and 70/75 nuts per kg. The I treatment had its nuts mostly represented in the size classes with the lower number of nuts per kg and with the highest frequencies. Furthermore, the highest differences, in terms of frequency, between the two treatments, were in the middle classes (e.g., 60/65) with I treatment showing higher frequencies compared to NI (Figure 4). On the contrary, NI trees showed lower frequencies compared to I in most of the classes (from 48/50 to 80/85 size classes) and exhibited a more uniform gaussian curve. NI trees had higher frequencies in the classes with the higher number of nuts per kg (from 85/90 to 100+ size classes).

4. Discussion

The cumulative precipitation occurred during November 2019 - October 2020 in Marradi was rather abundant if compared to the typical historical values (1999-2019 period) of the considered area (1232 and 898 mm, respectively, Table 1). In particular, in the November-March period, this difference was rather marked, since it rained 736 mm, while in 1999-2019 in the Marradi area typically rains 414 mm of cumulative rainfall, representing an increase of about 78%. These precipitations probably allowed a higher water storage in the soil, especially in the deeper soil layers, allowing an increase of water sources as a fundamental supply for the vegetative period. It is known that chestnut tree exploits its deep root system for absorbing water, especially in drought conditions (Martins, et al., 2010; Mota, et al., 2018c).

The higher water availability continued also during the summer period, with rainfall in July-August that in 2020 were almost the double (152.6 mm) of the typical mean values registered for the same month in the last 1999-2019 period (85 mm), thus providing most probably a stable and constant water supply also during the tree vegetative growth. Furthermore, summer temperatures were rather mild, with only 4 days (47, 48, 57, 59 DAFB) with T_{max} above 32 °C, that is considered a thermo-inhibition threshold temperature for Portuguese *C. sativa* cultivars (e.g., "Judia", "Longal") grown in Portuguese environments (Gomes-Laranjo et al., 2007, 2008). Portugal is known to have warmer and drier summers compared to the area studied in our research. Mota et al. (2018c) reported the following precipitations for July and August periods: 9.6, 37.5, 7.9 and 10.6 mm in 2013, 2014, 2015 and 2016, respectively.

Even the air relative humidity was rather high during the summer period with likely optimal RH values for not negatively affecting g_s and E (Araujo-Alves et al., 1993) (Figure 1). It is known that a suitable atmospheric humidity is among the major factor influencing chestnut physiological performances (Araujo-Alves et al., 1993).

The physiological parameters (A, E, g_s) measured after the first irrigation (58 DAFB) did not differ between trees of the two treatments. Probably at this time of the season chestnut trees were still utilizing water from the deeper soil layers, where the stored water was likely still high due to the winter soil replenishment. Chestnut trees mostly rely, in the summer season and especially during drought periods, on water available in deep soil layers, including permanent water tables (Martins et al., 2010). The chestnut tree root apparatus is indeed facilitated in exploring deep soil layer by the fractured nature of the mother stone (e.g., often sandstone). The small differences, found by Martins et al. (2010), in the predawn tree water status between irrigated and rainfed treatments, indicated the importance of deep roots (deeper than 80 cm) in extracting water during the night, since no differences, between these two treatments, were detected in the soil moisture at this depth. It should also be considered that the present study was carried out on an adult orchard that was never previously irrigated. Furthermore, physiological measurements (64 DAFB) were performed 6 days after the irrigation supply (58 DAFB) when the water content on the top soil layers was probably already decreased, especially in a soil with a sandy loam texture.

At 82 DAFB, the irrigation started to positively influence leaf gas exchanges with significant differences compared to the rainfed conditions (Figure 2). Mota et al. (2014), in accordance with the present study, found significant A differences between irrigated and non-irrigated treatments in a 20-year-old orchard, with an increase in photosynthetic rate of about 60-65% in the full irrigated treatment (10-14 μ mol CO₂ m⁻² s⁻¹) compared to the non-irrigated trees. The transpiration rate also was enhanced by the irrigation with an increase of 50% compared to the non-irrigated trees (Mota et al., 2014).

In a further study, Mota et al. (2018b) found that in August midday A was significantly higher in the irrigated than in non-irrigated treatment in both years of the study (2015 and 2016). Rainfed trees had the following A values: 7.1 and 5.6 μ mol CO₂ m⁻² s⁻¹ in 2015 and 2016, respectively. The irrigated trees showed instead higher A values: 9.6-9.9 and 7.4-7.3 μ mol CO₂ m⁻² s⁻¹ in 2015 and 2016, respectively. In the same study, the transpiration rate, although not significantly different between the treatments, was slightly reduced in the non-irrigated trees than in irrigated ones (1.5, 1.7-1.9 mmol H₂O m⁻² s⁻¹ for non-irrigated and irrigated, respectively, in 2015; and 1.8, 2.0-2.1 mmol H₂O m⁻² s⁻¹ for non-irrigated and irrigated, respectively, in 2015; and 1.8, 2.0-2.1 mmol H₂O m⁻² s⁻¹ for non-irrigated and irrigated, respectively, in 2016) (Mota et al., 2018b). Martins et al. (2010) also observed significant A differences, in 2003 season, between irrigated (8.88 μ mol CO₂ m⁻² s⁻¹) and non-irrigated (6.22 μ mol CO₂ m⁻² s⁻¹) treatments, while no differences were observed, by the same author, in 2005 and 2006 seasons.

The stomatal conductance (g_s) was also significantly improved by the irrigation (Figure 2). Unfortunately, comparisons with others chestnut studies could not be done due to the scarce literature found for this parameter. The g_s is highly dependent not only on soil water availability but also on the atmosphere relative humidity. Araujo-Alves et al. (1993) found that g_s , in some Portuguese chestnut cultivars, was positively correlated to RH. In the present study the high air humidity recorded in August and September was likely not limiting plant physiological performances, inferring that the higher g_s in the irrigated treatment was likely related to the higher soil water availability.

Midday Ψ_{stem} was significantly influenced by the irrigation treatment (Figure 3). The more positive Ψ_{stem} registered for I compared to the NI trees, suggest the positive effect of the irrigation on plant water relations. This data is in agreement with what reported by Mota et al. (2018b, c) who found that, in two consecutive years, irrigated chestnut trees had higher stem water potential compared to non-irrigated ones. More negative midday Ψ_{stem} values were registered in 2016 (-1.5 MPa for non-irrigated and -1.29/-1.30 MPa for irrigated treatments), the hotter year, compared to 2015 (-1.19 MPa for non-irrigated and -1.13/-1.10 MPa for irrigated treatments).

 Ψ_{leaf} was not influenced by the irrigation treatment (Figure 3). The quite unsettled weather condition at 82 DAFB could have affected and altered the midday Ψ_{leaf} readings. Indeed, Mota et al. (2014) found significantly higher midday Ψ_{leaf} values in irrigated (-1.2 to -1.6 MPa) than in non-irrigated trees (-1.7 to -2 MPa), in a 20 year old orchard. Martins et al. (2010) also found that midday Ψ_{leaf} was significantly higher for irrigated trees only in the driest years of the experiment (2003 and 2005).

These results suggest that, even on an adult chestnut orchard never managed with irrigation and under non-stressful weather conditions, the wetting of the top soil layers was able to enhance tree physiological performances. This highlights the importance of the upper roots (i.e., top soil layers) for water absorption during daylight (Mota et al., 2014).

Most of the nut quality traits measured in this study were positively influenced by irrigation (Table 2). The effect of irrigation likely emerged because irrigation was provided during the nut starch accumulation phase, that is a fundamental stage for plant productivity. Indeed, Chen et al. (2017) found, in *C. mollissima*, that nut started accumulating starch in the last phases of nut development (at 60 DAFB with a peak at 80 DAFB). The improved tree physiological performances in the irrigated treatment likely allowed higher carbohydrate synthesis to sustain nut growth. Carbohydrates (mainly starch) represent almost half of chestnut kernel fresh weight, being also the major component of its dry matter (Dinis et al., 2012). Furthermore, Perulli et al. (2020) reported that winter-spring and summer rainfall influence the overall burr absolute growth rate and consequently the final burr size. It is well known that, in chestnut, the availability of rain at the end of summer/beginning of autumn, seems to affect significantly chestnut development and productivity (Vigiani, 1941; Breisch et al., 1995; Bounous, 2002; Gomes-Laranjo et al., 2007; Vida Rural, 2017; Mota et al., 2018b).

In our study, irrigated trees had significantly higher nut weight and diameter than NI (Table 2). Mota et al. (2018b, c) found a significant increase in nut weight in the irrigated (77-89 nuts kg⁻¹) compared to non-irrigated (123 nuts kg⁻¹) trees only in the drier and hotter season (2016) of the two considered years (2015-2016). This result is also in agreement with Martins et al. (2011) who found bigger nuts in irrigated trees. Linhares et al. (2005) reported statistically significant differences for nut weight, between irrigated and non-irrigated treatments both in 2003 and 2004, while no statistical differences were found in fruit size. Similarly, Martins et al. (2005b), in two consecutive years, found bigger fruits in the irrigated treatments (11.05 and 12.37 g nut⁻¹, respectively for 2003 and 2004) compared to rainfed ones (9.75 and 11.14 g nut⁻¹, respectively for 2003 and 2004). The same authors, as in the present study, found a significative difference in terms of fruit diameter between the irrigated (2.88 cm) and non-irrigated (2.84 cm) trees, but only in one year (2003) (Martins et al., 2005b).

The difference in nut weight allowed to gain a higher commercial size class for I (65/70 nuts kg⁻¹) compared to the NI (70/75 nuts kg⁻¹) (Table 2). This result is encouraging because would permit to get a better quality nut (in term of size) and thus a higher profit for the farmers selling their products for the fresh market and for the industry (e.g., marron glace). The chestnut market tends to valorize mainly nut size (Martins et al., 2011). The positive effect of the irrigation was furtherly evidenced by the size class distribution (Figure 4) where I treatment had higher frequencies in the size classes with the lower number of fruits per kg (i.e., higher quality) compared to NI, which instead registered a higher frequency distribution in the classes with the higher number of fruits per kg (from 85/90 to 100+ nuts kg⁻¹).

As concern the nut dry matter, no significant differences were found between I and NI trees. This result agrees with Mota et al. (2018a). The nut dry matter values measured in our study were slightly higher than those recorded by Mota et al. (2018a), in 2015, with 45.3 and 45.8-46.4% for non-irrigated and irrigated treatments, respectively, while they were lower than those of 2016, 49.8 and 48.4-50.1% for non-irrigated and irrigated treatments, respectively. In any case, these dry matter values are in line with most of the studies conducted on Portuguese chestnut varieties (e.g., "Judia") (Ferreira-Cardoso et al., 2007; Dinis et al., 2011). Furthermore, the nut dry matter percentages found in this study were within the recommended value (> 40%) for nut conservation (Breisch, 1995), and this suggests that irrigation did not affect negatively the suitable nut water content for its conservation stability.

Tree yield, although showing an increase of 6 kg tree⁻¹ in the I treatment compared to NI, was not statistically different between the two treatments (Table 2). Also Mota et al. (2017, 2018a) did not find significative yield differences between non-irrigated (33-44 kg tree⁻¹) and irrigated (44-54 kg tree⁻¹) trees for two consecutive years (2015 and 2016), although showing slightly decreased yield in non-irrigated trees. Martins et al. (2005a, 2010, 2011) in most of their studies did not find yield differences between irrigated and non-irrigated trees in adult chestnut orchards (e.g., 39 years old). Adult chestnut plantations, as previously mentioned, are more likely dependent on the water content in the deep soil layers (for a deep root apparatus) than on that in the top soil layers that is mainly affected by irrigation. Indeed, only Jayne (2005) in an experimental trial with young chestnut trees (12 years old) observed the increasing of nut production with irrigation. Probably drier weather conditions both in winter (i.e., lower deep soil layers water refill) and during summer, would be able to significantly highlight the positive effect of irrigation practices on the yield of adult chestnut orchards.

Although chestnut productivity is highly related to the inter-annual weather (e.g., rainfall) variations (Martins et al., 2011), the present study highlights also how the irrigation practice is beneficial for a traditional orchard under non-stressful weather conditions. Indeed, the measured physiological parameters indicated that trees were not under water stress (e.g., below -1.2 MPa midday Ψ_{stem}). In any case, the phenotypical traits of chestnut cultivars, together with the environmental conditions of the different growing area, should be taken into account to improve chestnut physiological response to irrigation practices.

The irrigation management applied at a specific chestnut phenological stage (e.g., nut filling phase) using the actual availability water (often limited) could be a strategy to be adopted and integrated with the already experienced chestnut irrigation management studies (Martins et al., 2010, 2011; Mota et al., 2014, 2017, 2018b, 2018c), for both improving chestnut yields and saving water. To date, chestnut irrigation was mainly managed based on landowner knowledge or on the monitoring of the water status by the readings of predawn (-0.6 MPa irrigation threshold) or midday water potentials (-1.2 MPa irrigation threshold). These two latter methods, although being scientifically accurate, are complex for the frequent and laborious readings during the season (Mota et al., 2017). Furthermore, these methods are also quite difficult to be integrated and applied in a commercial orchard because they need specialized personnel for the reading and for the data interpretation. The integration of actual chestnut tree water needs together with its physiological processes, as expressed by burr growth, would be the optimal strategy to develop irrigation protocols for this species in areas with water limitations. Indeed, fruit growth may be used to monitor tree water status and thus to drive the irrigation management as already demonstrated for other fruit tree species (e.g., apple, pear and kiwifruit) (Morandi et al., 2017; Manfrini et al., 2019). However, different climatic conditions, soil type, and tree cultivars should be considered when adopting this irrigation approach. Chestnut cultivars are indeed well adapted to the environmental conditions of their area of origin (Alessandri et al., 2020). The knowledge of the physiological traits of the main cultivated chestnut ecotypes, still scarce for Italian ones, represents the starting point for the adoption of tailored and rational orchard management practices as irrigation is.

5. Conclusions

The overall results of this research showed how, in a traditional adult Italian chestnut orchard and in a year with no stressful weather conditions, the irrigation supplied during the nut filling phase (i.e., end of summer) positively enhanced chestnut leaf gas exchanges, tree water status, nut quality (e.g., size) and nut yield per tree. The use of irrigation will be increasingly essential to reach more regular and higher production over the years and to overcome the effect of climate change, especially when modern and specialized orchards will be planted. Further studies should be carried out in order to improve irrigation management in terms of water requirement, irrigation volumes and systems, applying smart solutions for the assessment of irrigation effects on burr growth during the season to further enhance chestnut productivity and quality. Funding: This research received no external funding.

Acknowledgement: Authors would like to thank all the Valgimigli family, in particular the farmer Dott. Alessandro Valgimigli, for their great effort, support and passion demonstrated in any phase of the research activities.

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

References

- Alessandri, S., Krznar, M., Ajolfi, D., Cabrer, A.M.R., Pereira-Lorenzo, S. and Dondini, L. (2020) 'Genetic diversity of castanea sativa mill. accessions from the tuscan-emilian apennines and emilia romagna region (Italy)', Agronomy, 10 (9), doi: 10.3390/agronomy10091319
- Araujo-Alves José Pedro L., Torres-Pereira M. Suzel B.C. and Torres-Pereira José M.G. (1993) 'Influence of atmospheric humidity and leaf water potential on stomatal conductance and transpiration in different chestnut tree cultivars', *Proceedings of the Interational Congress on Chestnut*. Spoleto, October 20-23.

Arpae (2021). Available at https://www.arpae.it/.

- Basile, B., Girona, J., Behboudian, M.H., Mata, M., Rosello, J., Ferré, M. and Marsal, J. (2012) 'Responses of "Chardonnay" to deficit irrigation applied at different phenological stages: Vine growth, must composition, and wine quality', *Irrigation Science* 30, 397-406. doi: 10.1007/s00271-012-0353-1
- Bounous, G. (2002) 'Frutteto di castagno', in: Bounous G. (ed.) *Il Castagno*. 1st edn. Bologna: Edagricole, pp. 71-129.
- Breisch, H., Boutitie, A., Reyne, J., Salesses, G. and Vaysse, P. (1995) 'Chataignes et marrons', Paris: CTIFL Centre Technique des Fruits et Legumes.
- Chen, L., Lu, D., Wang, T., Li, Z., Zhao, Y., Jiang, Y., Zhang, Q., Cao, Q., Fang, K., Xing, Y. and Qin, L. (2017) 'Identification and expression analysis of starch branching enzymes involved in starch synthesis during the development of chestnut (Castanea mollissima Blume) cotyledons', PLoS One, 12, pp. 1-15. doi: 10.1371/journal.pone.0177792
- Climate-Data (2021). Available at : <u>https://it.climate-data.org/</u>
- Decreto Ministeriale (1939) Norme speciali tecniche per l'esportazione delle castagne. Articolo 4, D.M 10-VII-1939 (XVII), Gazzetta Ufficiale del Regno D'Italia, N. 165.
- Dinis, L.T., Ferreira-Cardoso, J., Peixoto, F., Costa, R. and Gomes-Laranjo, J. (2011) 'Study of morphological and chemical diversity in chestnut trees (var. 'Judia') as a function of temperature sum', *CyTA - J. Food* 9, 192–199.
- Dinis, L.T., Peixoto, F., Ferreira-Cardoso, J. V., Morais, J.J.L., Borges, A.D.S., Nunes, F.M., Coutinho, J.F., Costa, R. and Gomes-Laranjo, J. (2012) 'Influence of the growing degree-days on chemical and technological properties of chestnut fruits (var. "Judia")', *CyTA-Journal Food*, 10, pp. 216-224. doi: 10.1080/19476337.2011.631713
- Ferreira-Cardoso, J. (2007) Valorização da castanha portuguesa, características tecnológicas e nutricionais. In: Gomes-Laranjo, J., Ferreira-Cardoso, J., Portela, E., Abreu, C.G. (Eds.), Castanheiros, 1st ed. *Vila Real*, pp. 282–345.
- Fideghelli, C. (2016) 'Castagno', in Bellini, E. and Morelli, D. (eds.) *Atlante dei fruttiferi autoctoni italiani* vol. II, Roma: MIPAAF, CREA, pp. 553-691.
- Gomes-Laranjo, J., Coutinho, J.P. Peixoto, F. and Araújo-Alves, J. (2007) 'Ecologia do castanheiro (C. sativa Mill.)' in Gomes-Laranjo, J., Ferreira-Cardoso, J., Portela, E. and Abreu, C.G. (eds.) Castanheiros. *Vila Real*: Universidade Trás-os-Montes e Alto Douro, pp. 109-150.
- Gomes-Laranjo, J., Coutinho, J. and Peixoto, F. (2008) 'Ecophysiological Characterization of C. sativa Mill.', *Acta Horticulturae*, pp. 99-106.

- Gomes-Laranjo, J., Dinis, L.T., Marques T., Mota, M., Carvalho, A., Pinto, T., Anjos, R., Martins, L., Marques, G., Gaspar, M.J., Gonzalez Pereira, M., Raimundo, F. and Ferreira-Cardoso, J. (2018)
 'Increasing chestnut resilience to climate change with innovative management practices', *Acta Horticulturae*, pp. 163-176. doi: 10.17660/actahortic.2018.1220.24
- Jayne, E. (2005) Châtaignier Optimisation des techniques d'irrigation. SEFRA/Chambre d'Agriculture de l'Ardè- che. [cit. 2017-01-29]. <u>http://rhone-alpes.synagri.com/synagri/pj.nsf/TECHPJPAR-CLEF/03131/\$File/irriga.pdf?OpenElement</u>
- Linhares, I., Martins A., Borges, O., Guedes. C. and Sousa. V., (2005) 'Effect of irrigation and soil management practices on fruit production and quality in chestnut orchards of northern Portugal', *Acta Horticulturae*. 693, ISHS.
- Manfrini, L., Zibordi, M., Pierpaoli, E., Losciale, P., Morandi, B. and Corelli Grappadelli, L. (2019) 'Development of Precision apple fruit growing techniques: Monitoring strategies for yield and high quality fruit production', *Acta Horticulturae*, 2019, 1261, pp. 191–197.
- Martins, A., Linhares, I., Raimundo, F., Coutinho, J.P., Gomes-Laranjo, J., Borges, O. and Sousa, V. (2005a) 'The importance of deep soil layers to supply water to agro-forestry systems: a case study of a mature chestnut orchard in northern Portugal', *Acta Horticulturae*. 663–670. doi: 10.17660/ActaHortic.2005.693.89
- Martins, A., Sousa, V., Guedes, C., Linhares, I. and Borges, O., (2005b) 'Effect of Irrigation and Soil Management Practices on Fruit Production and Quality in Chestnut Orchards of Northern Portugal', *Acta Horticulturae*. 701–706. doi: 10.17660/actahortic.2005.693.94
- Martins, A., Raimundo, F., Borges, O., Linhares, I., Sousa, V., Coutinho, J.P., GomesLaranjo, J. and Madeira, M. (2010) 'Effects of soil management practices and irrigation on plant water relations and productivity of chestnut stands under Mediterranean conditions', *Plant Soil*, 327, pp. 57-70. doi: 10.1007/s11104-009-0031-0
- Martins, A., Marques, G., Borges, O., Portela, E., Lousada, J., Raimundo, F. and Madeira, M. (2011) 'Management of chestnut plantations for a multifunctional land use under Mediterranean conditions: effects on productivity and sustainability', *Agroforestry Systems*, 81(2), pp. 175-189. doi: 10.1007/s10457-010-9355-2
- Morandi, B., Boselli, F., Boini, A., Manfrini, L., Corelli, L. (2017) 'The fruit as a potential indicator of plant water status in apple', *Acta Horticulturae*, 2017, 1150, pp. 83–90.
- Mota, M., Pinto, T., Marques, T., Borges, A., Raimundo, F., Veiga, V., Caço, J., Martins, A. and Gomes-Laranjo, J. (2014) 'Watering chestnuts orchards in Northeast Portugal Preliminary study.' *Acta Horticulturae* 1043, 129–133. doi: 10.17660/ActaHortic.2014.1043.16
- Mota, M., Pinto, T., Marques, T., Borges, A., Caço, J., Raimundo, F. and Gomes-Laranjo, J. (2017) 'Study on yield values of two irrigation systems in adult chestnut trees and comparison with nonirrigated chestnut orchard', *Revista de Ciencias Agrarias*, 41, 236-248.
- Mota, M., Pinto, T., Vilela, A., Marques, T., Borges, A., Caço, J., Ferreira-Cardoso, J., Raimundo, F. and Gomes-Laranjo, J. (2018a) 'Irrigation positively affects the chestnut's quality: the chemical composition, fruit size and sensory attributes', *Scientia Horticulturae*, 238, pp. 177-186. doi: 10.1016/J.SCIENTA.2018.04.047
- Mota, M., Marques, T., Pinto, T., Raimundo, F., Borges, A., Caço, J. and Gomes-Laranjo, J. (2018b) 'The effect of irrigation on chestnut physiology and production (Castanea sativa)', *Acta Horticulturae*, pp. 185-194. doi: 10.17660/ActaHortic.2018.1220.26
- Mota, M., Marques, T., Pinto, T., Raimundo, F., Borges, A., Caço, J. and Gomes-Laranjo, J. (2018c) 'Relating plant and soil water content to encourage smart watering in chestnut trees', *Agricultural Water Management* 203, 30–36. doi: 10.1016/j.agwat.2018.02.002
- Naor, A., Klein, I. and Doron, I. (1995) 'Stem Water Potential and Apple Size', *Journal of the American Society for Horticultural Science*, Issue 120, p. 577–582.
- Naor, A. (2006) 'Irrigation scheduling of peach Deficit irrigation at different phenological stages and

water stress assessment', Acta Horticulturae 713, pp. 339-349.

- Perulli, G.D., Boini, A., Bresilla, K., Morandi, B., Grappadelli, L.C. and Manfrini, L. (2020) 'Growth analysis of sweet chestnut burr in two seasons with differing weather conditions', *Italus Hortus* 27, 31–39. doi: 10.26353/j.itahort/2020.1.3139
- Torres-Ruiz, J.M., Perulli, G.D., Manfrini, L., Zibordi, M., Lopez Velascoo, G., Anconelli, S., Pierpaoli, E., Corelli-Grappadelli, L. and Morandi, B. (2016) Time of irrigation affects vine water relations and the daily patterns of leaf gas exchanges and vascular flows to kiwifruit (Actinidia deliciosa Chev.), *Agricultural Water Managenent* 166. doi: 10.1016/j.agwat.2015.12.012
- Turner, N. and Long, M. (1980) 'Errors Arising From Rapid Water Loss in the Measurement of Leaf Water Potential by the Pressure Chamber Technique', *Functional Plant Biology*, Issue 7, p. 527.
- Vernol, D. (2013) Eurocastanea.org. Available at: <u>http://www.eurocastanea.org/uploads/1/7/0/4/</u> 17040934/vernol presentation chataigne france 09 2013.pdf
- Vida Rural, R. (2017) Produtores de castanha pedem apoios para enfrentar ano de 'calamidade'. *Vida Rural.*
- Vigiani D. (1941) 'Influenza di alcuni fattori meteorologici sulla produzione del castagno'. *Atti Reale Accademia dei Georgofili*, XIX, pp. 3-10.
- Vigiani D. (1908) *Il Castagno*. Casale Monferrato. Tipografia e litografia Carlo Cassone. Capitolo III, Cure culturali Irrigazione, pp. 54-55.



© 2022 by the authors. Licensee Italian Society for Horticultural Science (Società di Ortoflorofrutticoltura Italiana; SOI), Sesto Fiorentino (Firenze), Italy. This work is an open access article distributed under a Creative Commons Attribution-NonCommercial (CC BY NC) 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/).