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Sweet cherry water relations and fruit production efficiency are affected by rootstock vigor.

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SUMMARY

Rootstock vigor is well known to affect yield and productive performance in many fruit crops and the dwarfing trait is often the preferred choice for modern orchard systems thanks to its improved productivity and reduced canopy volume. This work investigates the different physiological responses induced by rootstock vigor on cherry, by comparing shoot and fruit growth, water relations, leaf gas exchanges as well as fruit vascular and transpiration in/outflows of "Black Star" trees grafted on semi-vigorous (CAB6P) and on semi-dwarfing (Gisela™6) rootstocks. The daily patterns of stem (Ψ_{stem}), leaf (Ψ_{leaf}) and fruit (Ψ_{fruit}) water potential, leaf photosynthesis, stomatal conductance and transpiration, shoot and fruit growth, fruit phloem, xylem and transpiration flows were assessed both in pre- and post-veraison, while productivity and fruit quality were determined at harvest. At both stages, no significant differences were found on Ψ_{leaf} , photosynthesis, fruit daily growth rates as well as fruit vascular and transpiration flows, while trees on Gisela™6 showed lower shoot growth rates and lower Ψ_{stem} and Ψ_{fruit} than trees on CAB6P. The resulting decrease in stem-to-leaf Ψ gradient on Gisela™6 trees determined a reduction in shoot growth by decreasing shoot strength as sinks for water and carbohydrates. On the other hand, Gisela™6 fruit lowered their Ψ_{fruit} thanks to a higher osmotic accumulation and increased their competitiveness towards shoots, as confirmed by the higher productivity and fruit soluble solid content found at harvest for these trees. These results indicate that rootstock vigor alters resource competition between vegetative and reproductive growth, which can affect water use efficiency, yield, and fruit quality.

Keywords: Fruit growth, Leaf gas exchanges, Prunus Avium L., Sink strength, Water relations,

ABBREVIATIONS:

AGR: absolute growth rate; DAFB: days after full bloom; RGR: relative growth rate; SSC: soluble solids content; Ψ_{stem} : stem water potential; Ψ_{fruit} : fruit water potential; Ψ_{leaf} : leaf water potential

1. INTRODUCTION

In the past, cherry orchards have been characterized by low and/or medium planting densities (≈ 500 trees/ha) on high vigorous rootstocks like seedlings (*P. avium* Mazzard and *P. mahaleb*), the hybrid "Colt" (*P. Avium* x *P. Pseudocerasus* Lindl.) or the sour cherry clones of the CAB series (Lang, 2000; Lugli, 2011). However, in different cherry productive regions, the current trend is to choose rootstocks allowing medium/high densities (800-1200 trees/ha) to anticipate production, facilitate tree management (pruning, harvest etc.) due to their reduced canopy size and improve the orchard productive efficiency (Lang, 2000; Lang, 2005; Lang et al., 2014; Hrotkò and Rozpara, 2017).

Currently, the most used dwarfing rootstocks in high density orchards are the interspecific hybrids of the series Gisela™ (mostly Gisela™ 6 and Gisela™ 5), and more recently the series PHL and Pi-Ku (Lang, 2000; Lugli, 2011).

It is well known how dwarfing rootstocks are characterized by a strong reduction in the xylem hydraulic conductivity in correspondence of the grafting point where often callus and meristematic tissues tend to proliferate, in response to different levels of disaffinity between the two living tissues (Olmstead et al. 2006; 2010). Such disaffinity leads to a progressive decrease in the carbohydrate transport toward the roots, with consequent lower root development and reduced absorption capacity (Olmstead et al., 2010). The rootstock can also affect the scion xylem anatomy as trees on dwarfing rootstocks tend to show lower vessel diameters and higher frequencies (Olmstead et al. 2006; Gonçalves et al. 2006a; Ljubojevuc et al., 2013). A lower hydraulic conductivity in the xylem leads to a lower water transport capacity and tends to reduce the scion vegetative growth, also due to a decrease in the stem water potential and to a consequent reduction in leaf nutritional status and gas exchanges (Gonçalves et al. 2006a, 2006b; Edwards et al., 2014). Despite stomatal conductance tends to be reduced (Edwards et al., 2014), trees on dwarfing rootstocks usually show higher photosynthesis/stomatal conductance ratios, and thus a higher photosynthetic efficiency (Gonçalves et al. 2006b). In cherry, they have also been found to reduce the meristematic activity of their growing shoots due to a change in the gene expression both in the scion and at the graft union level (Prassinos et al., 2009).

Rootstock vigor can also affect productivity and quality of the production (Lang, 2000; Neilsen et al., 2016), due to variations in the hydraulic anatomy and in the water relations of the scion (Peschiutta et al., 2013), although crop load and thus leaf/fruit ratio, seems to be the major driver affecting fruit development and final fruit quality (Whiting and Lang, 2004; Neilsen et al., 2016). Regarding fruit quality, Gonçalves et al. (2006b) showed how thanks to their better water status, vigorous rootstocks usually show a higher fruit size, also because the combination between autofertile cultivars and dwarfing rootstocks may induce excessive crop loads (Bassi 2005). However, despite the wide differences on yield and quality of the production, it is not clear yet how vigor affects the developmental physiology of fruit growth and the evolution of the quality traits within the fruit.

Cherry fruit development during the season is described by the double-sigmoid model typical of stone fruit (Dejong and Gudriann, 1989; Gibeaut et al., 2017). Regardless of the phenological stage, fruit growth always results from the balance between vascular and transpiration in/outflows (Fishman and Génard, 1998), therefore, knowledge on the mechanisms underpinning fruit growth would represent key information to understand the productive efficiency of a given scion/rootstock combination. The availability of automatic sensors for the continuous monitoring of fruit diameter variations (Morandi

et al., 2007a), has allowed to study the biophysical and physiological mechanisms of fruit growth in different species (Lang, 1990; Morandi et al., 2007b; 2010; 2014), including cherry. In this regard, Brüggenwirth et al. (2016) reported how during the first stages of fruit development, at about 40 DAFB, cherry fruit growth was mostly sustained by the xylem, that accounted for about 85% of the total daily inflows, while the phloem contribution was relatively low. However, these inflows were mostly lost by epidermis transpiration (accounting for about 85% of the total daily inflows). As fruit developed, the contribution of the phloem flow progressively increased, while xylem flow decreased accordingly so that, after veraison, cherry fruit growth was almost totally sustained by the phloem (Brüggenwirth et al. 2016).

However, despite specific knowledge is available on the effect of the different scion/rootstocks combinations on fruit quality and yield, to date it is not clear how rootstock vigor affects the source/sink water and carbon relations at whole canopy level and whether the physiological mechanisms of fruit growth, and thus the evolution of the fruit quality traits during the season, change depending on the rootstock. In fact, despite the use of dwarfing rootstocks usually leads to an improvement in yield, productive efficiency and (not always) fruit quality, results are highly variable depending on variety, orchard age, irrigation management, soil type and cultivation environment and the reasons underpinning such variability are not completely clear yet. This work studies how rootstock vigor can affect tree water relations, leaf gas exchanges and the daily vascular flows underpinning sweet cherry fruit growth in pre- and post-veraison.

2. MATERIALS AND METHODS

2.1 Plant material and experimental set up.

The study was carried out in 2016 on 16 cherry trees, cv. Black Star. Half of these trees were grafted on the semi-vigorous rootstock "CAB6P" *(Prunus Cerasus)* (-10-20% vigor compared to seedling - Lugli et al., 2011) and the other half on the semi-dwarfing rootstock "Gisela ™ 6" *Prunus cerasus* (cv *Schattenmorelle* × *Prunus canescens*) (-50-60% vigor compared to seedling – Lugli, 2011).

The trial was carried out in the Po Valley, at the experimental farm of the University of Bologna (Cadriano, Bologna, Italy). Trees were at their 12^{th} leaf, spaced 4.5x0.9m, with a density of 2470 trees ha^{-1} and trained as V. The orchard was managed according to standard cultural practices in terms of fertilization, thinning and pruning. Irrigation was managed according to the Irrinet irrigation scheduling system, developed and made available over the Internet by the "Consorzio per il Canale Emiliano Romagnolo (CER)" of the Emilia-Romagna Region (www.irriframe.it). A weather station located near the orchard collected the environmental parameters for the use of the software.

Full bloom occurred on April 1st and fruit were harvested on June 1st, 61 days after full bloom (DAFB).

For each rootstock, the daily patterns of leaf, stem and fruit water potential and of leaf gas exchanges were determined at 47 and 55 DAFB. Fruit daily growth and vascular and transpiration flows were also monitored, in the period from 27 to 32 DAFB and from 50 to 54 DAFB. Veraison occurred around 50 DAFB so all physiological measurements were carried out both in pre- (1st date) and in post-veraison (2nd date). Shoot and fruit growth were monitored at regular time intervals during the whole season. At harvest, average fruit weight, yield and soluble solid content were also assessed.

2.2 Seasonal fruit and shoot growth

During the season, the growth of 64 fruit and 48 shoots, on 8 trees per rootstock, randomly selected, was monitored on a weekly basis, using a digital caliper and a meter, respectively. For each fruit, diameter (D) data were converted to weight (FW) by the following conversion equation (Eq.1);

Eq 1: $FW(g) = a * D(mm)^b$

where a and b were 0.0021 and 2.5431. This equation was obtained by regressing diameter and weight data of a large number (above 300) of Black Star cherry fruit picked over several seasons, from the same orchard. The R^2 of the relationship was >0.99.

2.3 Water relations

Stem (Ψ_{stem}), leaf (Ψ_{leaf}) and fruit (Ψ_{fruit}) water potentials were monitored at predawn and at 9.00, 12.30 and 16.30 hours, on 4 trees per treatment, using a Scholander (Soilmosture Equipment Corp. Santa Barbara, U.S.A.) pressure chamber. Ψ_{leaf} was measured on one well exposed shoot leaf per tree, on 4 trees per treatment following Turner and Long (1980). Ψ_{stem} was measured on the same trees: one leaf per tree placed in the inner part of the canopy, very close to the main stem, was chosen and covered with aluminium foil at least 90 minutes prior to measurement to allow equilibration with the stem, according to the methodology described by McCutchan and Shackel (1992) and by Naor *et al* (1995). Similarly, Ψ_{fruit} was measured on one fruit per tree, with a total of 4 fruit per treatment.

For every measurement time, stem-to-leaf and stem-to-fruit Ψ gradients were calculated as the difference between Ψ_{stem} and Ψ_{leaf} and between Ψ_{stem} and Ψ_{fruit}, respectively. At all recording times and for all parameters, means (±SE) were then computed.

148 *2.4 Leaf gas exchanges*

149 The daily patterns of leaf gas exchanges (net photosynthesis, transpiration and stomatal conductance)
150 were measured almost in correspondence with the water potential measurements (at 9:00, 13:00 and
151 16:00 hour), using an open circuit infra-red gas exchange system fitted with a LED light source (Li-
152 COR 6400, LI-COR, Lincoln, Nebraska, USA). Measurements were carried out on one leaf per tree
153 on 4 trees per treatment. During each measurement, light intensity was maintained constant setting
154 the LED light source to the natural irradiance experienced by the leaves immediately before the
155 measurements.

156

157 *2.5 Fruit growth, vascular and transpiration flows*

158 Daily fruit growth, phloem inflow, xylem in/outflow and transpiration outflow were determined on
159 trees grafted on CAB6P and Gisela ™ 6, following Lang (1990). This method assumes fruit diameter
160 variation in a finite time interval as the result of the algebraic sum among phloem, xylem and
161 transpiration flows. Vascular and transpiration flows are then calculated as the difference between
162 the diameter variations of intact, girdled and detached fruit. This calculation is based on the further
163 assumptions that: (i) xylem flow is not affected by girdling (Van der Wal et al., 2017) and (ii)
164 transpiration rate is not affected by detachment. Fishman *et al*., 2001 report how assumption can lead
165 to some systematic errors, causing under- and over-estimation of phloem and xylem flows,
166 respectively. However, these errors seem to be limited to specific times during the day and, to date,
167 this is the only method which allows to estimate vascular and transpiration flows in the field, at short
168 times scales and on a statistically sound number of samples.

169 The fruit diameter variations were monitored at 15 minute intervals by custom-built gauges interfaced
170 with a wireless data-logger system. The gauges consisted of a light, stainless steel frame supporting
171 a variable linear resistance transducer (Megatron Elektronik AG & Co., Munchen, Germany) (Fig 1).
172 Temperature effects on the frame and the sensor were tested and showed negligible errors under
173 normal field conditions (Morandi *et al.* 2007b). The wireless data-logger system (Wi-Net s.r.l.
174 Cesena, Italy) (Giorgetti et al., 2014) to which the gauges were connected was composed of wireless
175 nodes, located on the topmost part of the pillar, at the beginning of the rows, to send a better signal
176 to a central network node, which acted as a gateway towards the internet, through a general packet
177 radio service (GPRS) modem.

178 Fruit vascular and transpiration flows were determined over 4-6 days of measurement starting at 27
179 and at 50 DAFB, for measurements carried out before and after veraison, respectively. For each
180 period, diameter variations over time were simultaneously monitored on 8 representative, well
181 exposed fruit placed on both sides of the row. Six of these fruit were subjected to the following

sequence of conditions: “intact” (with normal vascular connections), “girdled” (with the phloem connection severed) and “detached” (with all vascular connections severed). Phloem connections were severed by girdling the branch at both sides of the fruit pedicel insertion. In “detached” fruit the peduncle surface was covered with glue to avoid any water loss, and fruit were hung in their original position using thin wire. Fruit were monitored for one/two days on each of the “intact” and “girdled” conditions and only for one day on the “detached” condition, then, for each fruit, data collected on days with the same condition were averaged. Detached fruit were monitored for a shorter period of time (1 day) to avoid excessive dehydration of the tissue, which could lead to underestimate fruit transpiration. These measurements were carried out during periods with clear, sunny conditions. During these periods, 2 of the 8 fruit per treatment were continuously monitored in intact conditions and served as controls to verify that fruit daily growth rate and pattern did not change significantly during the period of measurement.

For each fruit, diameter data were converted to weights using Eq1.

The relative changes in fresh weight in a given time interval (t) were then calculated on each of the three conditions: normal (N), girdled (G) and detached (D), and phloem (P), xylem (X) and transpiration (T) flows were computed using the following equations:

Eq. 2: $P_t = N_t - G_t$

Eq. 3: $X_t = G_t - D_t$

Eq. 4: $T_t = D_t$

Fruit growth rate, phloem, xylem and transpiration flows were expressed both as weight changes per whole fruit (g fruit^{-1}) and per unit of fruit weight (g g^{-1}). For all parameters (fruit growth, phloem, xylem and transpiration flows) fresh weight (FW) and changes per whole fruit (AGR) were calculated at daily time intervals (t = day) using the following equation:

Eq. 5: $AGR_{t1} = (FW_{t1}-FW_{t0})/(t_1-t_0)$

Similarly, FW changes per unit of fruit weight (RGR) were calculated at daily time intervals (t =day), using the following equation:

Eq. 6: $RGR_{t1} = (FW_{t1}-FW_{t0})/(t_1-t_0)* FW_{t0}$

215 At each recording time, data from the 6 fruit per treatment measured were averaged and standard
216 errors were computed for all the parameters considered.
217
218 *2.6 Harvest, yield and fruit quality*
219 At 61 DAFB, the 16 trees used for the experiments (8 trees per treatment) were harvested and, for
220 each tree, total yield and average fruit weight (g) were determined. In addition, soluble solids content
221 (SSC) by was determined on 15 fruit per tree, on 4 trees. SSC was measured on a few juice drops by
222 refractometry.
223
224 *2.7 Statistical analysis*
225 On all dates considered, all parameters monitored were analyzed as a completely random design: for
226 the shoot and fruit growth, the water relation parameters ($\Psi_{stem,}$ Ψ_{leaf} and Ψ_{fruit}, stem-to-leaf and stem-
227 to-fruit Ψ gradient), the leaf gas exchanges (leaf photosynthesis, stomatal conductance and
228 transpiration), the daily fruit growth, vascular and transpiration flows, the harvest yield, fruit weight
229 and SSC, the two rootstocks were compared using a Student's t-test.
230
231 3. RESULTS
232 *3. 1 Seasonal shoot and fruit growth*
233 Since the beginning of the measurements, trees on CAB6P showed longer shoots compared to
234 Gisela™6 with initial lengths of 15.6±1 and 8.3±0.5 cm, respectively (Fig 2a). For both rootstocks,
235 shoot seasonal growth pattern showed higher growth rates at the beginning of the season, followed
236 by a general decrease in growth towards harvest (Fig 2a). During the initial stages, CAB6P shoots
237 showed growth rates peaks of about 1±0.2 cm/day (compared to the 0.7±0.3 cm/day of Gisela™ 6)
238 and reached almost twice the length of those on Gisela™ 6, at harvest (Fig 2a). On the contrary,
239 Gisela™ 6 ended up with shorter shoots, characterized by very low growth rates at veraison, in
240 correspondence with the period of maximum fruit growth (Fig. 2b).
241 For the two rootstocks, seasonal fruit growth showed the typical double sigmoid pattern characterized
242 by periods of high growth rates in correspondence with cell division and cell expansion. Despite a
243 slight difference recorded at 25-30 DAFB, both rootstocks maintained similar fruit growth rates
244 during the season, which were lower during the pit hardening stage, until 35 DAFB, and increased
245 afterwards, reaching values of ca. 0.37 g fruit^{-1} day^{-1} at 50-55 DAFB, in correspondence with fruit
246 cell expansion (Fig. 2b).
247
248 *3.2 Water Relations*

In both dates, no difference was recorded in pre-dawn Ψ_{stem} and Ψ_{fruit} between the two rootstocks. However, as time passed by, trees on Gisela™ 6 reached and maintained significantly lower Ψ_{stem} during the day, compared to CAB6P, with minimum values of -0.8±0.01 and -0.4±0.01 MPa at 47 DAFB and of -0.86±0.04 and -0.42±0.01MPa at 55 DAFB, for Gisela™ 6 and CAB6P, respectively (Fig 3a, c). Ψ_{leaf} never showed statistical differences between the two rootstocks, except at 55 DAFB, 9:00 hour, when Gisela™ 6 leaves showed slightly lower values compared to CAB6P (Fig 3c). Unlike leaves, Ψ_{fruit} was more affected by the rootstock, with fruit on Gisela™ 6 maintaining lower Ψ_{fruit} values compared to fruit on CAP6P. These differences appeared already at 47 DAFB, becoming more evident at 55 DAFB, with pre-dawn values of about -1.53±0.03 and -1.12±0.05 MPa for Gisela™ 6 and CAB6 fruit, respectively (Fig 3b, d).

On both dates, trees on Gisela™ 6 maintained lower stem-to-leaf Ψ gradients during the day, compared to CAB6P, where these gradients increased from the morning to the afternoon, reaching values of almost -1 MPa at 16:30 (Fig 4). On the contrary, except at 47 DAFB at 9:00 hour, similar stem-to-fruit Ψ gradients were maintained during the day, between the two rootstocks, with values around -1 MPa both at 47 and 55 DAFB (Fig 4).

3.3 Leaf gas exchanges and water use efficiency

CAB6P leaves showed a higher stomatal conductance at 9:00 and 16:00 hour, with values of 0.30±0.03 and 0.22±0.04 mol m^{-2} s^{-1}, respectively (Fig 5b). At the same hours, Gisela™ 6 leaves showed a decrease of about 30% and 50%, respectively (Fig 5b). Despite such an important decrease in stomatal conductance, these leaves did not show a significant reduction in photosynthesis, with values of 13.7±0.9 and 11.3±0.8 μmol CO_2 m^{-2} s^{-1} at 9:00 and 16:00 hour, respectively (Fig 5a). On the contrary, the daily trend in leaf transpiration followed changes in stomatal conductance with significantly higher water losses from CAB6P leaves, that reached values of about 4.3±0.6 mmol H_2O m^{-2} s^{-1} at 9:00 hour (Fig5c).

At 55 DAFB, the two rootstocks maintained similar photosynthesis and stomatal conductance at 9:00 and 12:00 hour, while at 16:30 hour Gisela™ 6 leaves were subjected to a 25% and a 50% reduction compared to CAB6P, respectively (Fig 6a, b). Water losses by transpiration maintained steady values during the day in Gisela™ 6 leaves, while CAB6P showed important increases in leaf transpiration that reached values of 6.4±0.2 mmol H_2O $m^{-2}s^{-1}$, corresponding to almost the double amount of water transpired by Gisela™ 6 leaves, at the same time of day (Fig 6c).

Water use efficiency, expressed as the amount of CO_2 fixed per water transpired (μmol CO_2 / mmol H_2O) was much higher for Gisela™ 6 both at 47 and 55 DAFB, with values that were ca. 30% higher than CAB6P, regardless of the date and time of day (Fig 5d and 6d).

3.4 Fruit growth and vascular flows

Fruit growth showed similar patterns between the two rootstocks, both when expressed on a specific (g g^{-1} d^{-1}) and on a whole fruit (g $fruit^{-1}$ d^{-1}) basis. Fruit relative growth rate (RGR) maintained steady values from 30 to 50 DAFB (ca. 30-40 mg g^{-1} d^{-1}) (Fig. 7a), while fruit absolute growth rate (AGR) widely increased during fruit development, reaching values of about 212±30 and 230±6 mg $fruit^{-1}$ d^{-1} for fruit on Gisela™ 6 and on CAB6P, respectively (Fig. 7b).

With increasing fruit size (from 2 to 8 g at the beginning and at the end of the season, respectively), fruit specific transpiration decreased from about -100 mg g^{-1} d^{-1} at 30 DAFB (2012) to -5 mg g^{-1} d^{-1} at 50 DAFB (Fig. 7c), with no apparent differences between the two rootstocks. A similar pattern was followed by specific xylem flow (Fig. 7e) which decreased accordingly. Specific phloem flow maintained steady values for both rootstocks (Fig. 7g). On a whole fruit basis, both xylem and transpiration flows decreased their daily amounts from 30 to 50 DAFB, with no statistical differences between rootstocks. On the contrary, phloem flow showed an important increase, passing from 54±23 and 55±22 mg $fruit^{-1}$ d^{-1} at 30 DAFB to 156±44 and 210±6 mg $fruit^{-1}$ d^{-1} at 50 DAFB, for Gisela™ 6 and CAB6P, respectively (Fig. 7h).

For both rootstocks, the relative contribution of the xylem and phloem flow to fruit growth was about 80 and 20% at 30 DAFB and changed to ca. 25 and 75% at 50 DAFB, respectively. Cherry fruit lost 70 and 20 % of their daily inflows by epidermis transpiration, at 30 e 50 DAFB, respectively.

3.5 Harvest Yield and fruit quality

Harvest data showed average yields of 2.59 and 1.99 kg/tree for trees grafted on Gisela™ 6 and on CAB6P, respectively, while similar average fruit weight (ca. 8 g/fruit) were obtained for the two rootstocks. Despite yield was not significantly different between the two rootstocks, these values indicate a higher crop load on Gisela™6 trees. Soluble solids content was higher for fruit on Gisela™ 6, with values of 19.5 °Brix, against the 17.18 °Brix recorded from CAB6P fruit (Table 1).

4. DISCUSSION

As reported in literature (Gonçalves et al. 2006a; Ljubojevuc et al. 2013,), rootstock vigor deeply affected the scion vegetative growth, with higher shoot lengths and growth rates on trees grafted on CAB6P, from the beginning of the season, until harvest (Fig. 2a). On the contrary, shoots on the more dwarfing rootstock Gisela™6 grew slowly, with minima in growth velocity in correspondence with the fruit cell expansion stage, the period of maximum fruit growth (Fig. 2a). The slower growth of Gisela™6 shoots suggests a shift in the relative resource partitioning towards fruit sinks which at this

317 stage need high amounts of water and carbohydrates to sustain their growth. CAB6 shoots continued
318 their growth also in correspondence with fruit cell expansion, the period of maximum fruit growth
319 rates, leading to a higher final vegetative growth, which is typical of vigorous rootstocks (Gonçalves
320 et al. 2006a, 2006b) (Fig 2a). On the other hand, rootstock vigor did not affect average fruit weight
321 during most of the growing season and at harvest. In fact, even if some differences in fruit growth
322 were recorded during cell division, trees on the two rootstocks maintained similar fruit growth rates
323 during the season, which were slower in correspondence with pit hardening and increased during cell
324 expansion, reaching maximum velocities (ca. 0.35 g $fruit^{-1}$ d^{-1}) at 50-55 DAFB, in correspondence
325 with veraison (Fig. 2b). Therefore, the semi-dwarfing rootstock Gisela™ 6 sustained the same fruit
326 growth of CAB6P, despite the much higher crop load and harvested yield (Table 1). These results are
327 in accordance with Ayala and Lang (2018) who reported how fruit from trees on Gisela™ 6 become
328 extremely strong sinks during the period of most rapid fruit growth. Other studies also confirm how
329 the higher productive efficiency of dwarfing rootstock tends not to compromise fruit size in cherry
330 (Lang, 2000; Bassi, 2005; Withing and Lang, 2004). Accordingly, daily vascular flows both
331 expressed as a specific contribution and on a whole fruit basis, did not show differences between
332 rootstocks, for any of the flows considered (phloem, xylem and transpiration) (Fig. 7), in agreement
333 with the similar seasonal fruit growth patterns and final fruit size, recorded for the two rootstocks
334 (Table 1).
335 Fruit vascular flows data confirm what reported by Brüggenwirth et al. (2016) and shows how, during
336 the initial stage of fruit development, cherry fruit growth is characterized by high water in/outflows
337 by xylem and transpiration, while the phloem contribution only accounts for about 20% of the total
338 daily inflows (Fig. 7). Later in the season, fruit water exchanges from the tree to the atmosphere
339 decrease, together with the xylem and transpiration flows. At the same time, the phloem contribution
340 increases and sustains fruit growth for about 80% in correspondence with fruit cell expansion (Fig 7).
341 These results suggest how the mechanism of cherry fruit growth changes deeply with fruit
342 development, leading to a progressive decrease in fruit surface conductance (Knoche et al., 2001) and
343 to a likely partial xylem dysfunctionality (Brüggenwirth and Knoche, 2015), as it happens in apple
344 (Lang, 1990), or to a lack in the necessary water potential gradients within the xylem vessels, which
345 on its turn can be a consequence of the reduced fruit transpiration. However, the relatively high stem-
346 to-fruit Ψ gradients recorded in this work during fruit cell expansion stage (Fig 4) suggest how this
347 latter hypothesis might be unlikely.
348 The fact that the vascular flows sustaining fruit growth are not affected by vigor can be explained by
349 the water relations and leaf gas exchange data recorded during the experiment (Fig 3-6). In fact, Ψ
350 data show how the rootstock deeply affects tree water status, with Ψ_{stem} reaching much more negative

values on Gisela™ 6 trees, compared to CAB6P (Fig 4). The lower Ψ_{stem} indicates the difficulty of dwarfing rootstocks to meet their canopy transpiration demand due: i) to the lower absorption capacity of their root system (which is less developed) and ii) to the lower hydraulic conductivity in the grafting point and in the scion (Gonçalves et al. 2005, 2006). As flows of water and carbohydrates move following Ψ gradients within the vascular system (Munch, 1930; Patrick, 1990), the more negative the Ψ_{stem}, the higher will be the need for shoots and fruit to decrease their Ψ in order to attract water and carbohydrate resources towards themselves. Despite the reduction in Ψ_{stem}, Gisela™ 6 leaves maintained similar Ψ_{leaf} to CAB6P (Fig. 3), resulting in lower stem-to-leaf Ψ gradients (Fig 4) during most of the day and thus in a lower capacity to attract water. The same mechanisms can apply to shoot tips, which might then lack the necessary turgor for their growth.

On the contrary, Gisela™6 fruit showed a better adaptation to the lower water availability thanks to a decrease in their Ψ_{fruit}, that allowed them to maintain stem-to-fruit Ψ gradients similar to CAB6P (Fig 4). As fruit transpiration flows are similar between the two rootstocks (Fig 7c,d), the lower fruit water potentials recorded for Gisela™6, especially as the fruit gets closer to maturity (Schumann et al., 2014) (Fig 3 b, d), could be due to specific mechanisms of osmotic adjustment (i.e. a higher solutes accumulation, probably in the apoplast) (Patrick, 1990) allowing fruit to attract more water and carbohydrates towards themselves, thus becoming sinks more competitive compared to shoot leaves. This hypothesis is supported by the higher soluble solid concentration found in Gisela™6 fruit at harvest (Table 1). In fact, soluble solid concentration has been found to be highly related to fruit osmotic potential (Winkler and Knoche, 2018), thus sustaining the hypothesis of an osmotic adjustment in fruit on dwarfing rootstocks. The relative change in the stem-to-leaf and in the stem-to-fruit Ψ gradients hereby reported contributes to explain the reduced vegetative growth typical of dwarfing rootstocks (Fig 2a), together with the maintenance of non-limiting fruit growth rates, as confirmed by the daily data for xylem and phloem flows, which are not affected by the rootstock (Fig 7). In addition, the leaf capacity to fix carbon was not significantly affected by the rootstock, except in one case, in the late afternoon, at 55 DAFB (Fig. 6a), despite leaves on CAB6P showed a much higher stomatal conductance compared to Gisela™ 6 during the day (Fig 6b). This difference could partly depend on the higher stem-to-leaf Ψ gradient recorded on CAB6P and thus on the higher capacity of these leaves to attract water (Fig. 4) which, by consequence, led to extremely high water losses by transpiration (Fig 5c and 6c) and to a lower water use efficiency (Fig 5d and 6d), at times of high VPD.

In our environmental conditions, the lower stomatal conductance on Gisela™ 6 trees does not appear to be limiting for photosynthesis and still allows the tree to fix enough carbon to sustain its fruit growth at similar rates to CAB6P. Furthermore, it prevents Gisela™6 leaves to lose high amounts of

water via transpiration, mainly during the afternoon hours, thus allowing a constantly higher WUE compared to the more vigorous rootstock (Fig. 6d). However, the reduced photosynthesis of Gisela™6 trees occurring at times of higher VPDs, which were recorded only occasionally in our conditions (such at 55 DAFB, in the late afternoon) (Fig 6a), might become a major limitation in more stressful environments, where the cumulative day by day effect of reduced carbon assimilation due to drought stress, over weeks, might significantly reduce the tree productive efficiency. This may explain the difficulties of applying dwarfing rootstocks in environments characterized by high evapotranspiration requirements and water scarcity.

CONCLUSIONS

Results reported in this paper provide some insights on how rootstock vigor can alter sweet cherry productive efficiency. According to our results, the physiological reasons of the higher productive efficiency of cherry trees on Gisela™6 can be summarized as follows:

i) When water is non-limiting, trees on dwarfing rootstocks maintain enough photosynthetic capacity to fully sustain their fruit growth. This occurs thanks to stomatal conductance values that, although reduced in comparison with more vigorous rootstocks, are still not limiting for photosynthesis.

ii) Changes in whole canopy water relations decrease the sink strength of shoots and lead to a shift in the relative partitioning of water and carbohydrates towards fruit sinks. This seems to occur thanks to an adaptation capacity of fruit that, unlike leaves, are more able to lower their Ψ_{fruit} to keep up with the lower Ψ_{stem} typical of dwarfing rootstocks. On the other hand, high-vigor rootstocks appear to physiologically shift towards higher vegetative sink strength thanks to the relatively higher Ψ gradient between stem and leaves.

Results also show a higher leaf water use efficiency in Gisela™6 trees. To maintain this efficiency a careful irrigation management might be important as trees on dwarfing rootstocks may be more sensitive to water scarcity, especially at times and in environments with high evapotranspiration requirements. In an environmental context where water is a limited resource, these data are particularly interesting, although further studies are needed to evaluate the actual possibility to use water more efficiently in orchard on dwarfing rootstocks, which are known to be more subjective to water stress.

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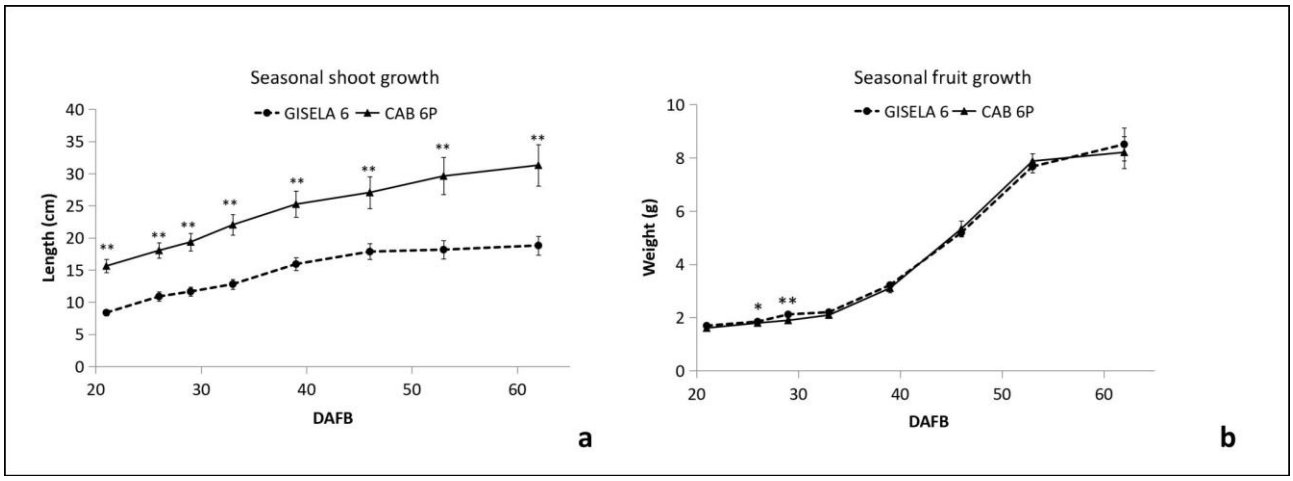
549 FIGURE LEGENDS

550

551 FIGURE 1 Gauge for the continuous monitoring of fruit growth of ‘Black Star’ sweet cherry at 55
552 DAFB.

553



554

555 FIGURE 2: Seasonal shoot (a) and fruit (b) growth pattern of ‘Black Star’ sweet cherry trees grafted
556 on CAB6P (continuous line) and Gisela™ 6 (dashed line) rootstocks. Each point represents the mean
557 (±SE) of 48 shoots and 64 fruit, respectively. Statistical comparison between rootstocks with
558 Student’s t test. *:P<0.05 **:P<0.01

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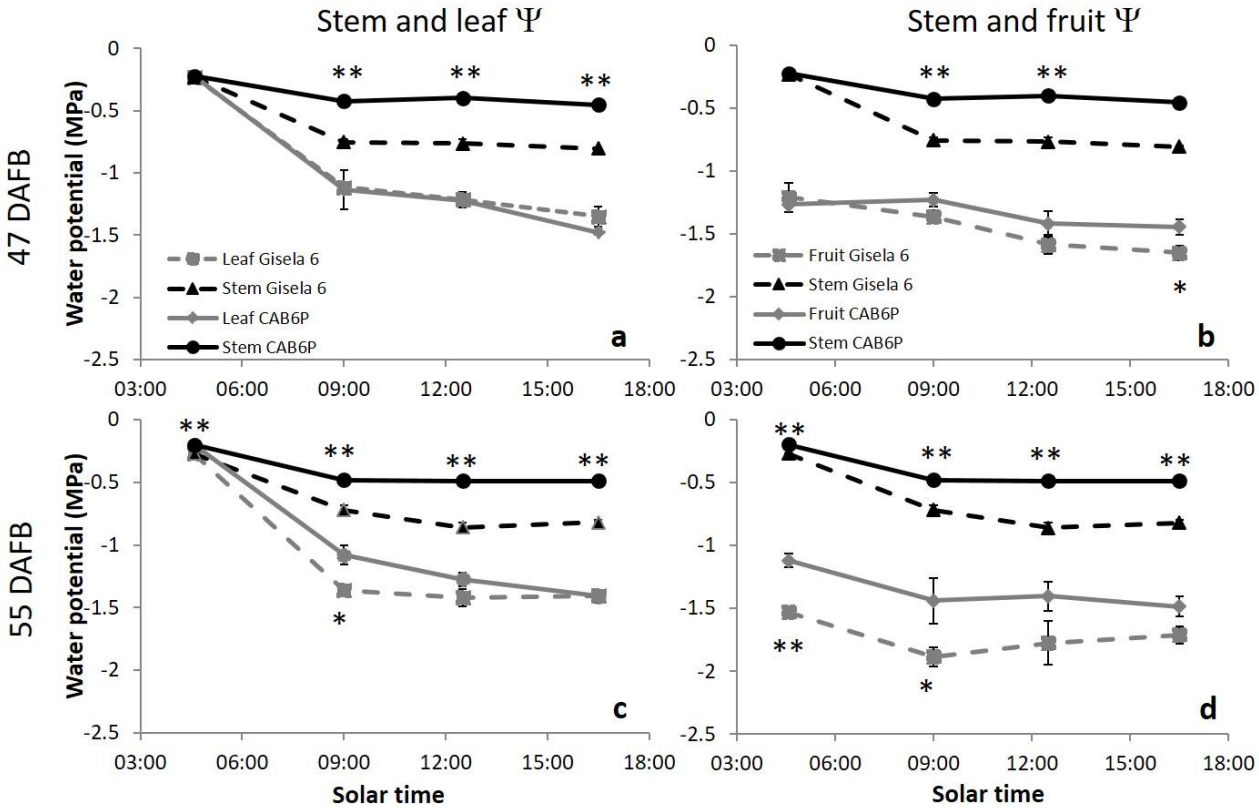
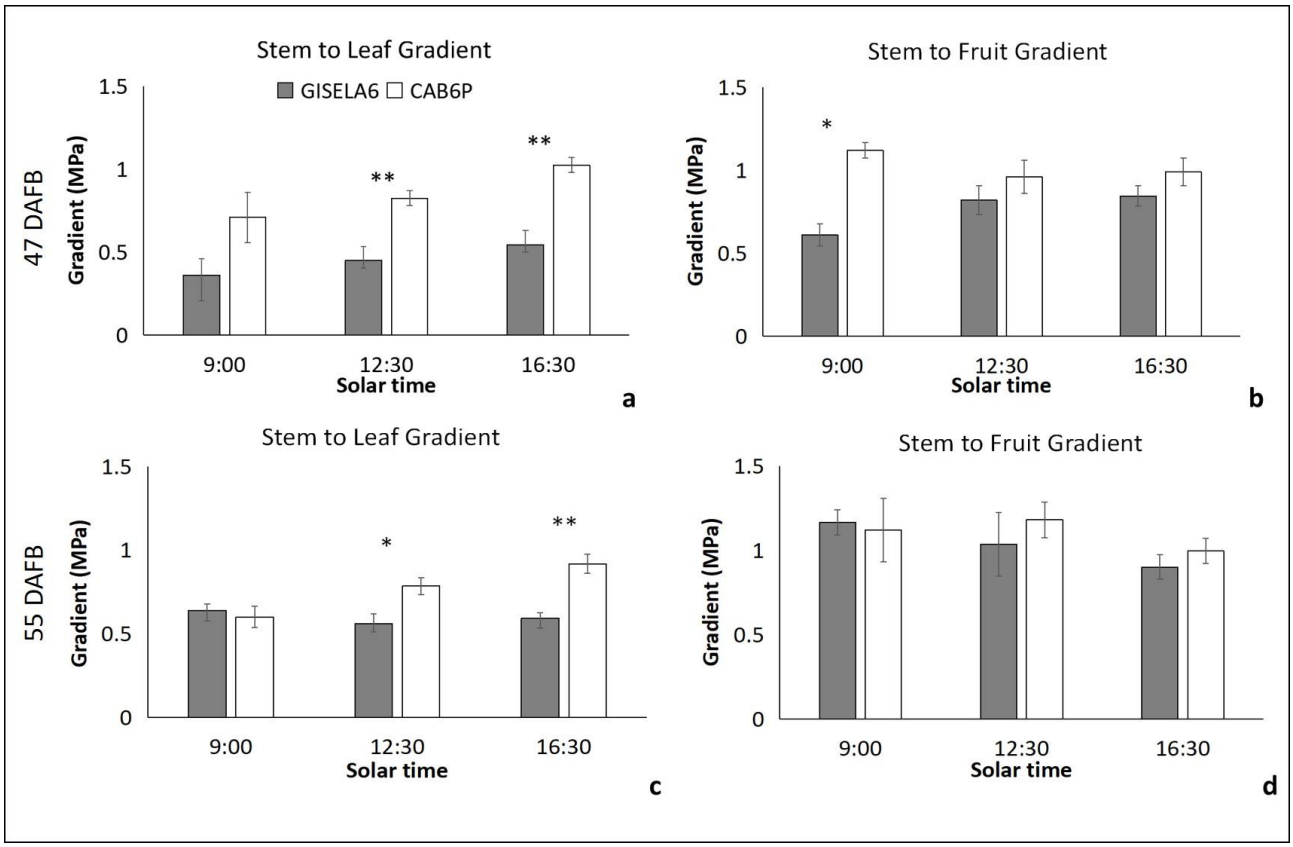


562

563 FIGURE 3 Diurnal pattern of stem (black lines) and leaf (grey lines) (a, c) and of stem (black lines)
564 and fruit (gray lines) water potentials (b, d) measured on 'Black Star' sweet cherry trees grafted on
565 CAB6P (continuous lines) and on Gisela™6 (dashed lines), at 47 (a, b) and at 55 (c, d) DAFB. Each
566 point represents the mean (±SE) of 4 measurements. Statistical analysis with Student's t test. *P<0.05
567 **P<0.01



568

569 FIGURE 4. Stem-to-leaf (a, c) and stem-t- fruit (b, d) Ψ gradients calculated for 9:00, 12:30 and
570 16:30 hour, at 47 DAFB and at 55 DAFB on 'Black Star' sweet cherry trees grafted on CAB6P (grey
571 bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean (±SE) of 4 replicates.
572 Statistical comparison between rootstocks with Student's t test. *:P<0.05 **:P<0.01

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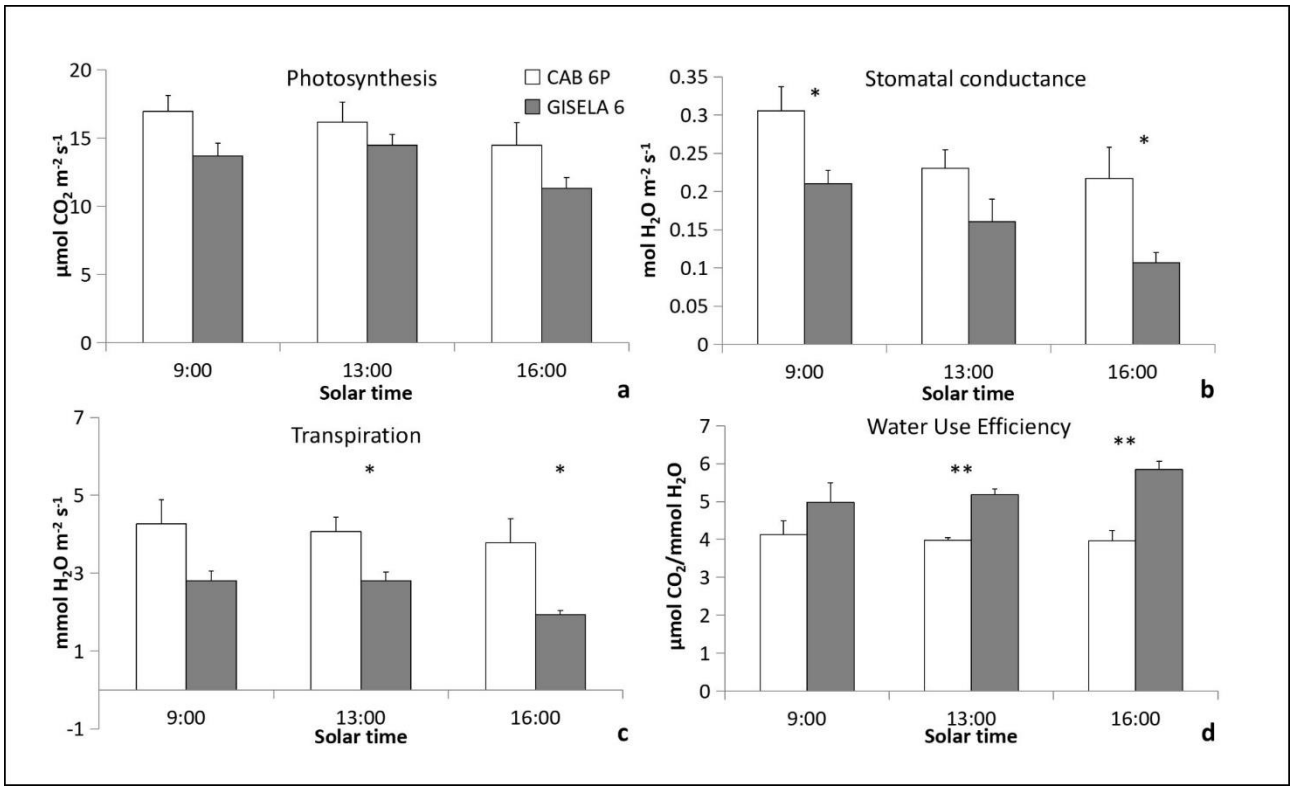

574

575 FIGURE 5: Leaf net assimilation rate (a), stomatal conductance (b), leaf transpiration (c) and water
576 use efficiency (WUE) recorded at 9:00, 13:00 and 16:00 hour, at 47 DAFB for 'Black Star' sweet
577 cherry trees on CAB6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean
578 (±SE) of 4 replicates. Statistical comparison between rootstocks with Student's t test. *:P<0.05
579 **:P<0.01

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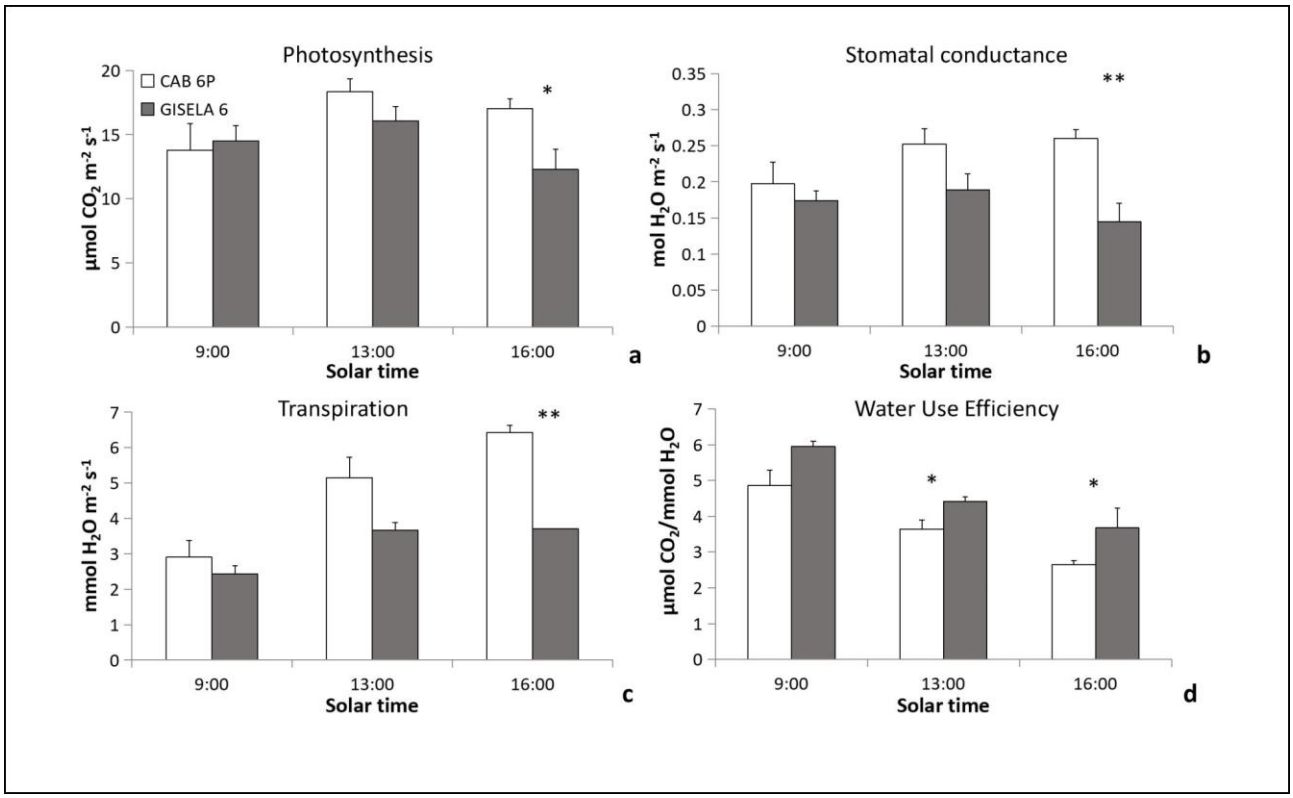
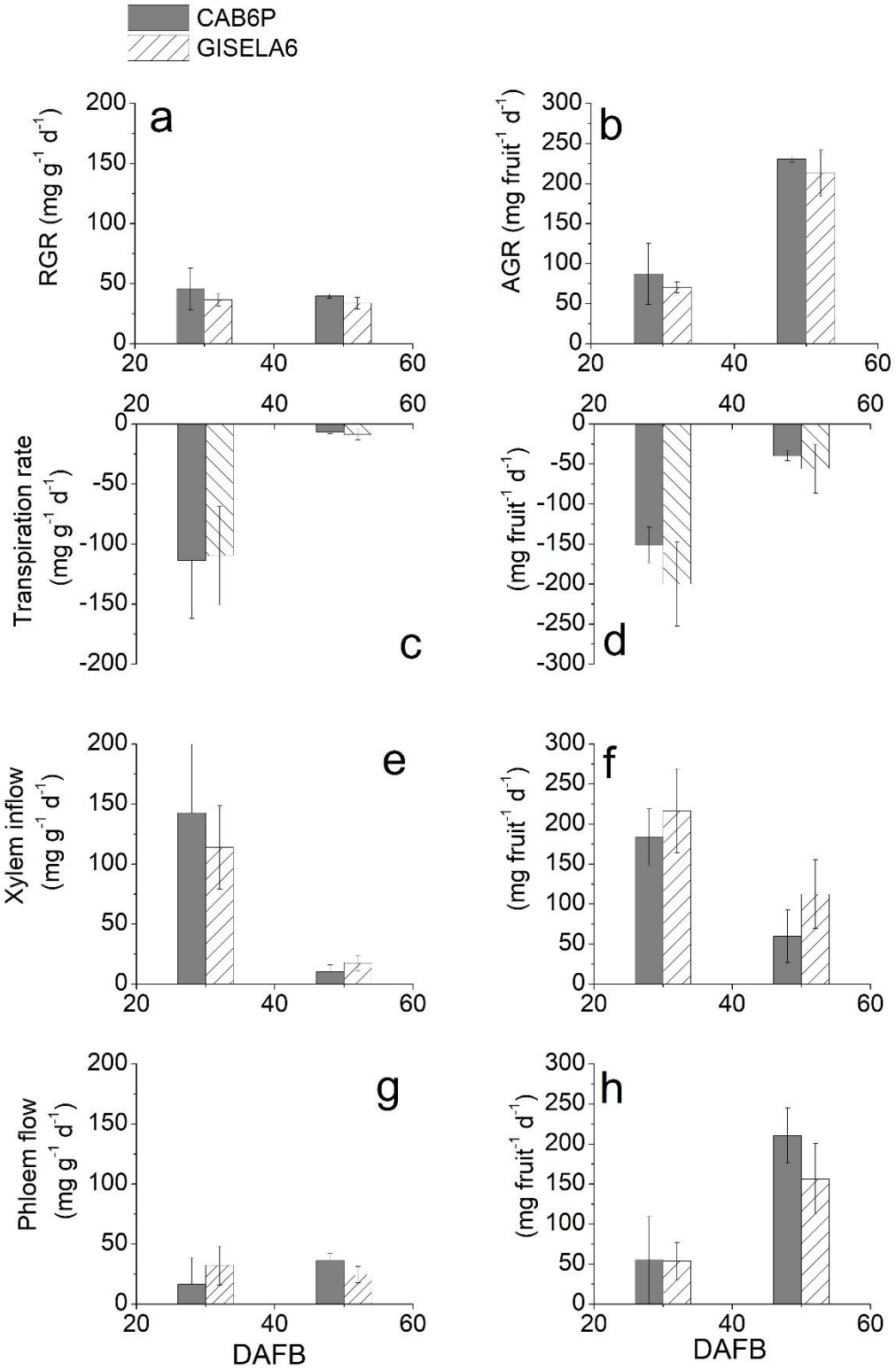


FIGURE 6: Leaf net assimilation rate (a), stomatal conductance (b), leaf transpiration (c) and water use efficiency (WUE) recorded at 9:00, 13:00 and 16:00 hour, at 55 DAFB for 'Black Star' sweet cherry trees on CAB 6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean (±SE) of 4 replicates. Statistical comparison between rootstocks with Student's t test. *:P<0.05 **:P<0.01



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588 FIGURE 7: Mean (± SE) relative (RGR) (a) and absolute (AGR) (b) growth rates, specific (c) and
589 whole fruit transpiration (d), specific (e) and whole fruit xylem flow (f), specific (g) and whole fruit
590 phloem flow (h) from/to cherry fruit of 'Black Star' sweet cherry trees grafted on CAB6P (grey bars)
591 and on Gisela™6 (dashed bars) rootstocks, at 30 and 50 DAFB. Data are the mean of at least 6 fruit.
592 No statistical difference was found between treatments using a Student's t test.

593

	Gisela™ 6	CAB6P	
Yield (kg/tree) ± SE	2.59 ± 0.27	1.99 ± 0.34	
SSC (°Brix) ± SE	19.54 ± 0.27	17.18 ± 0.32	*
Fruit weight (g) ± SE	8.5 ± 0.6	8.2 ± 0.7	

TABLE 1: Yield, soluble solids content (SSC) and mean fruit weight at harvest for 'Black Star' sweet cherry trees grafted on CAB 6P and Gisela ™ 6 rootstocks. Each bar represents the mean of 4 trees and of 15 fruit (±SE). Statistical comparison between rootstocks with Student's t test. *:P<0.05