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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 12th 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.

2.4 Leaf gas exchanges

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

2.5 Fruit growth, vascular and transpiration flows

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

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

Fruit vascular and transpiration flows were determined over 4-6 days of measurement starting at 27 and at 50 DAFB, for measurements carried out before and after veraison, respectively. For each period, diameter variations over time were simultaneously monitored on 8 representative, well 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: $RGR_{t1} = (FW_{t1}-FW_{t0})/(t_1-t_0)* FW_{t0}$

At each recording time, data from the 6 fruit per treatment measured were averaged and standard errors were computed for all the parameters considered.

2.6 Harvest, yield and fruit quality

At 61 DAFB, the 16 trees used for the experiments (8 trees per treatment) were harvested and, for each tree, total yield and average fruit weight (g) were determined. In addition, soluble solids content (SSC) by was determined on 15 fruit per tree, on 4 trees. SSC was measured on a few juice drops by refractometry.

2.7 Statistical analysis

On all dates considered, all parameters monitored were analyzed as a completely random design: for the shoot and fruit growth, the water relation parameters ($\Psi_{stem,}$ Ψ_{leaf} and Ψ_{fruit}, stem-to-leaf and stem-to-fruit Ψ gradient), the leaf gas exchanges (leaf photosynthesis, stomatal conductance and transpiration), the daily fruit growth, vascular and transpiration flows, the harvest yield, fruit weight and SSC, the two rootstocks were compared using a Student's t-test.

3. RESULTS

3. 1 Seasonal shoot and fruit growth

Since the beginning of the measurements, trees on CAB6P showed longer shoots compared to Gisela™6 with initial lengths of 15.6±1 and 8.3±0.5 cm, respectively (Fig 2a). For both rootstocks, shoot seasonal growth pattern showed higher growth rates at the beginning of the season, followed by a general decrease in growth towards harvest (Fig 2a). During the initial stages, CAB6P shoots showed growth rates peaks of about 1±0.2 cm/day (compared to the 0.7±0.3 cm/day of Gisela™ 6) and reached almost twice the length of those on Gisela™ 6, at harvest (Fig 2a). On the contrary, Gisela™ 6 ended up with shorter shoots, characterized by very low growth rates at veraison, in correspondence with the period of maximum fruit growth (Fig. 2b).

For the two rootstocks, seasonal fruit growth showed the typical double sigmoid pattern characterized by periods of high growth rates in correspondence with cell division and cell expansion. Despite a slight difference recorded at 25-30 DAFB, both rootstocks maintained similar fruit growth rates during the season, which were lower during the pit hardening stage, until 35 DAFB, and increased afterwards, reaching values of ca. 0.37 g fruit^{-1} day^{-1} at 50-55 DAFB, in correspondence with fruit cell expansion (Fig. 2b).

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 stage need high amounts of water and carbohydrates to sustain their growth. CAB6 shoots continued their growth also in correspondence with fruit cell expansion, the period of maximum fruit growth rates, leading to a higher final vegetative growth, which is typical of vigorous rootstocks (Gonçalves et al. 2006a, 2006b) (Fig 2a). On the other hand, rootstock vigor did not affect average fruit weight during most of the growing season and at harvest. In fact, even if some differences in fruit growth were recorded during cell division, trees on the two rootstocks maintained similar fruit growth rates during the season, which were slower in correspondence with pit hardening and increased during cell expansion, reaching maximum velocities (ca. 0.35 g fruit^{-1} d^{-1}) at 50-55 DAFB, in correspondence with veraison (Fig. 2b). Therefore, the semi-dwarfing rootstock Gisela™ 6 sustained the same fruit growth of CAB6P, despite the much higher crop load and harvested yield (Table 1). These results are in accordance with Ayala and Lang (2018) who reported how fruit from trees on Gisela™ 6 become extremely strong sinks during the period of most rapid fruit growth. Other studies also confirm how the higher productive efficiency of dwarfing rootstock tends not to compromise fruit size in cherry (Lang, 2000; Bassi, 2005; Withing and Lang, 2004). Accordingly, daily vascular flows both expressed as a specific contribution and on a whole fruit basis, did not show differences between rootstocks, for any of the flows considered (phloem, xylem and transpiration) (Fig. 7), in agreement with the similar seasonal fruit growth patterns and final fruit size, recorded for the two rootstocks (Table 1).

Fruit vascular flows data confirm what reported by Brüggenwirth et al. (2016) and shows how, during the initial stage of fruit development, cherry fruit growth is characterized by high water in/outflows by xylem and transpiration, while the phloem contribution only accounts for about 20% of the total daily inflows (Fig. 7). Later in the season, fruit water exchanges from the tree to the atmosphere decrease, together with the xylem and transpiration flows. At the same time, the phloem contribution increases and sustains fruit growth for about 80% in correspondence with fruit cell expansion (Fig 7). These results suggest how the mechanism of cherry fruit growth changes deeply with fruit development, leading to a progressive decrease in fruit surface conductance (Knoche et al., 2001) and to a likely partial xylem dysfunctionality (Brüggenwirth and Knoche, 2015), as it happens in apple (Lang, 1990), or to a lack in the necessary water potential gradients within the xylem vessels, which on its turn can be a consequence of the reduced fruit transpiration. However, the relatively high stem-to-fruit Ψ gradients recorded in this work during fruit cell expansion stage (Fig 4) suggest how this latter hypothesis might be unlikely.

The fact that the vascular flows sustaining fruit growth are not affected by vigor can be explained by the water relations and leaf gas exchange data recorded during the experiment (Fig 3-6). In fact, Ψ 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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FIGURE LEGENDS

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

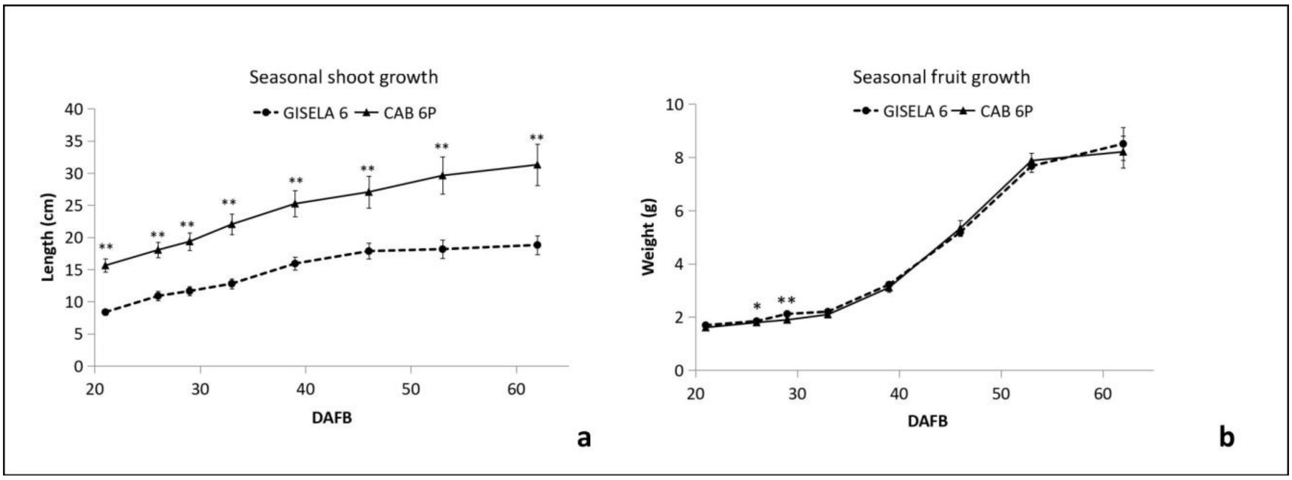

FIGURE 2: Seasonal shoot (a) and fruit (b) growth pattern of ‘Black Star’ sweet cherry trees grafted on CAB6P (continuous line) and Gisela™ 6 (dashed line) rootstocks. Each point represents the mean (±SE) of 48 shoots and 64 fruit, respectively. Statistical comparison between rootstocks with Student’s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

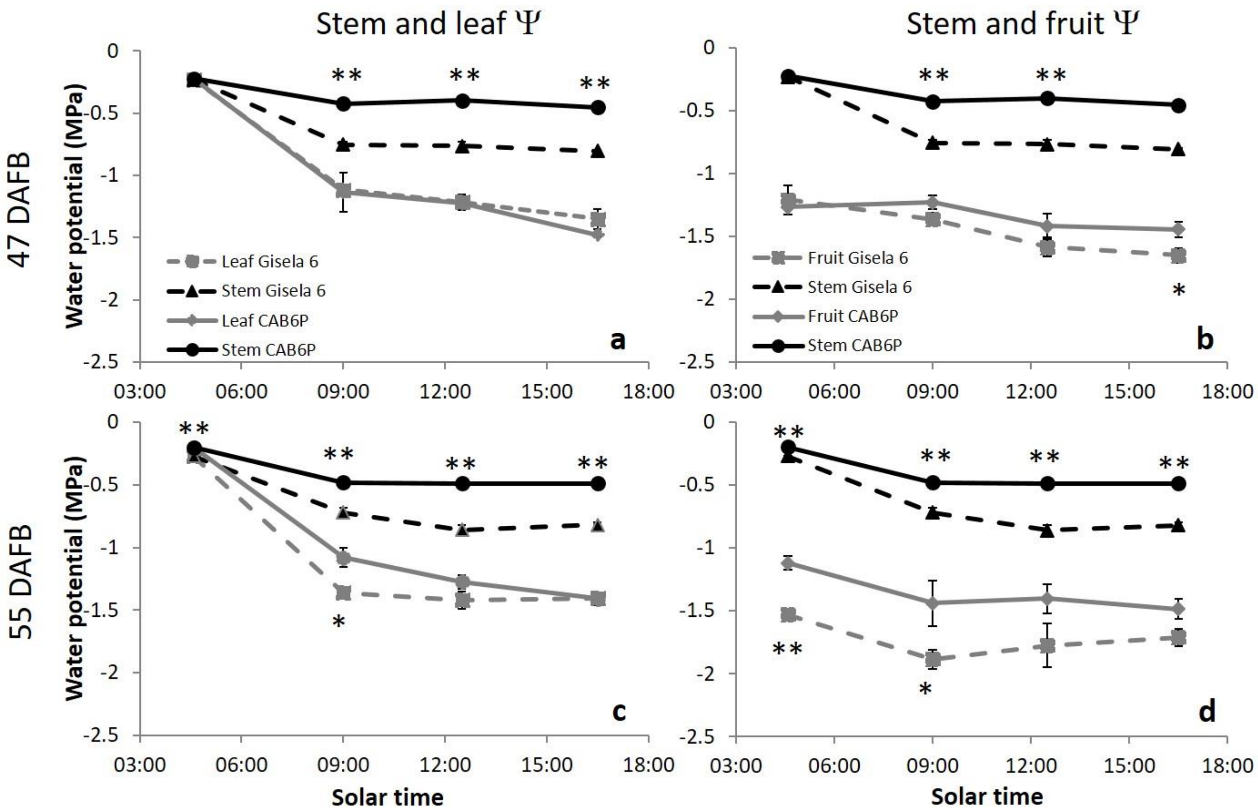

FIGURE 3 Diurnal pattern of stem (black lines) and leaf (grey lines) (a, c) and of stem (black lines) and fruit (gray lines) water potentials (b, d) measured on ‘Black Star’ sweet cherry trees grafted on CAB6P (continuous lines) and on Gisela™6 (dashed lines), at 47 (a, b) and at 55 (c, d) DAFB. Each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

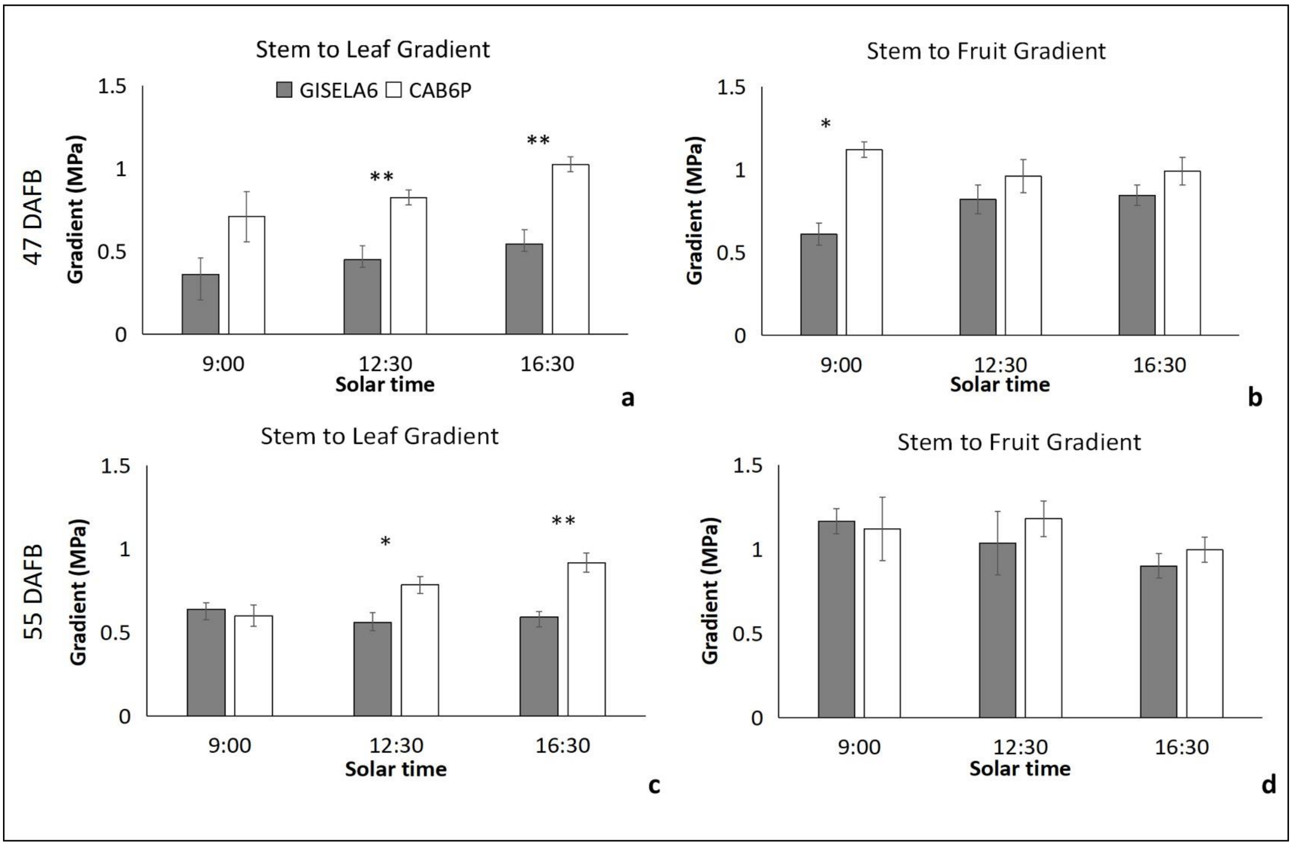

FIGURE 4. Stem-to-leaf (a, c) and stem-t- fruit (b, d) Ψ gradients calculated for 9:00, 12:30 and 16:30 hour, at 47 DAFB and at 55 DAFB on 'Black Star' sweet cherry trees grafted on CAB6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean (±SE) of 4 replicates. Statistical comparison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

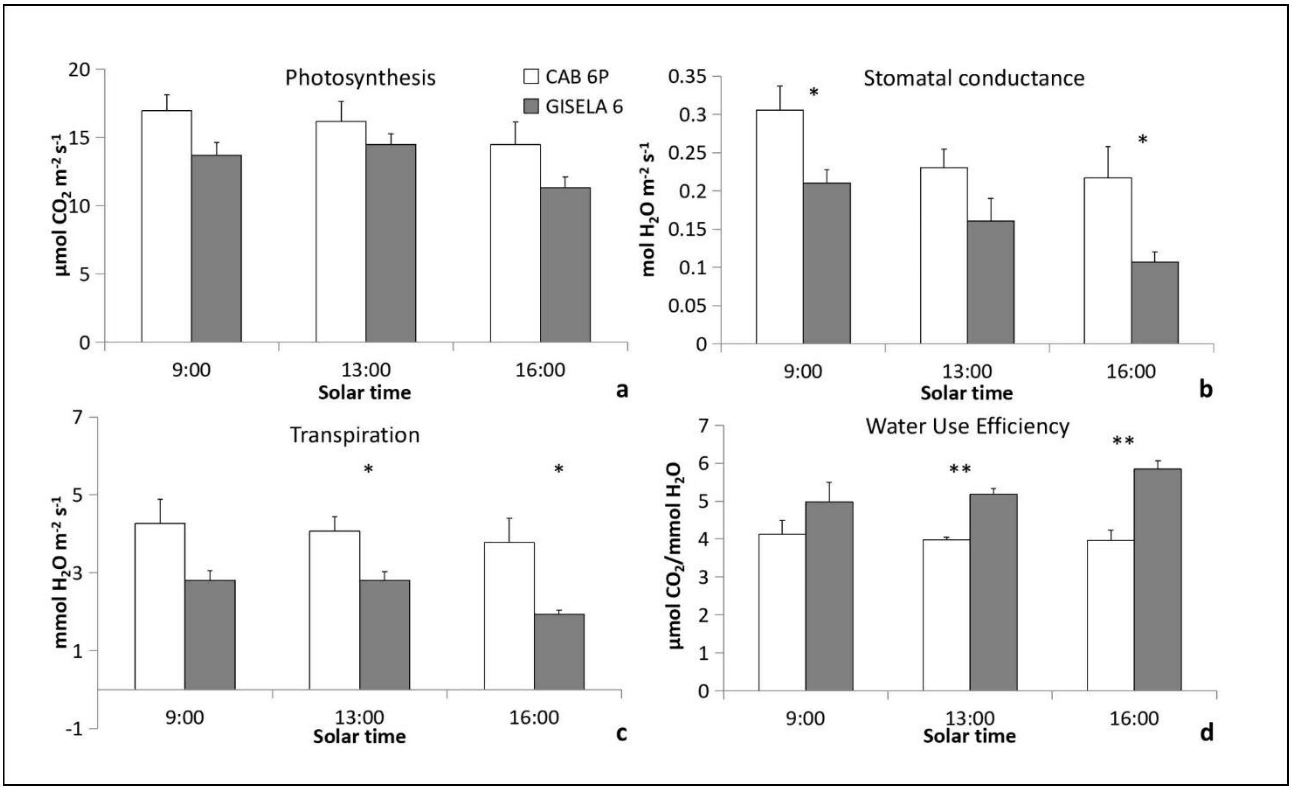

FIGURE 5: Leaf net assimilation rate (a), stomatal conductance (b), leaf transpiration (c) and water use efficiency (WUE) recorded at 9:00, 13:00 and 16:00 hour, at 47 DAFB for 'Black Star' sweet cherry trees on CAB6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean (±SE)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

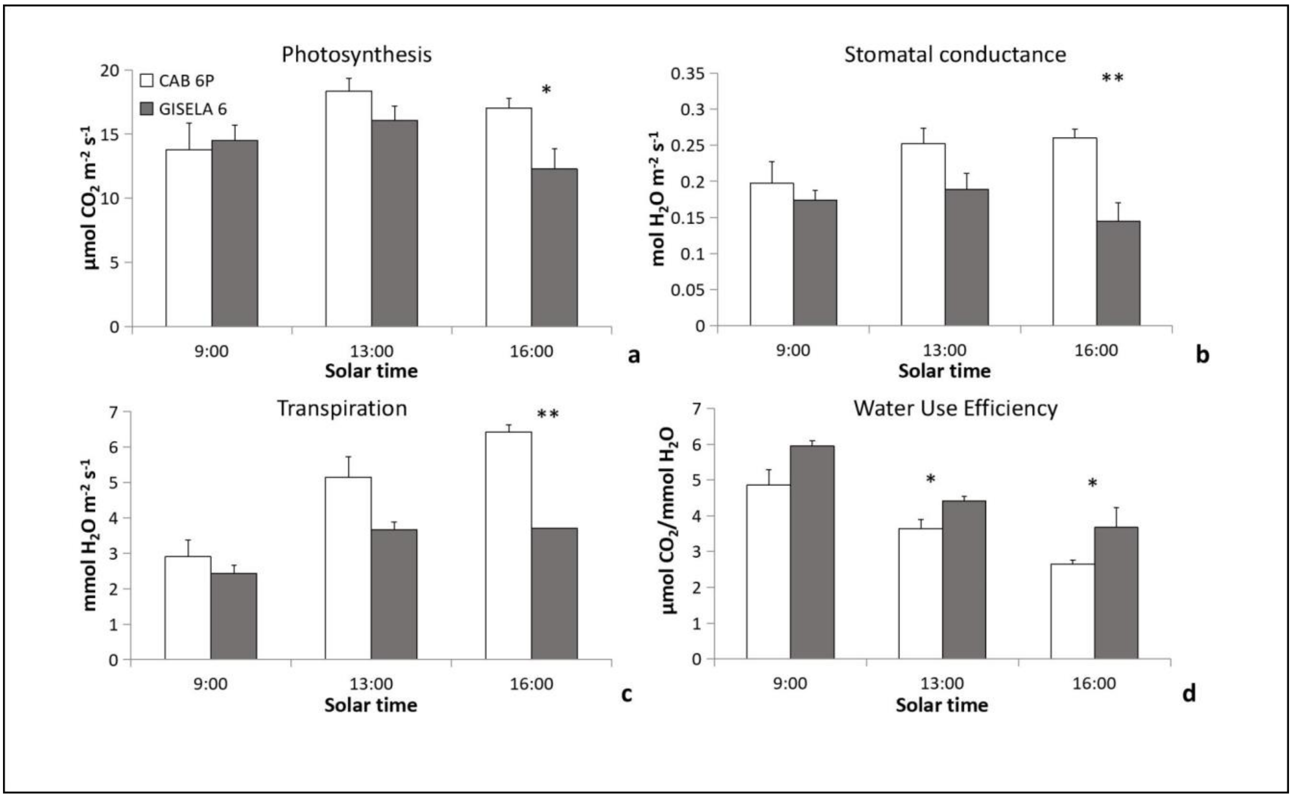

FIGURE 6: Leaf net assimilation rate (a), stomatal conductance (b), leaf transpiration (c) and water use efficiency (WUE) recorded at 9:00, 13:00 and 16:00 hour, at 55 DAFB for 'Black Star' sweet cherry trees on CAB 6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the mean (±SE)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

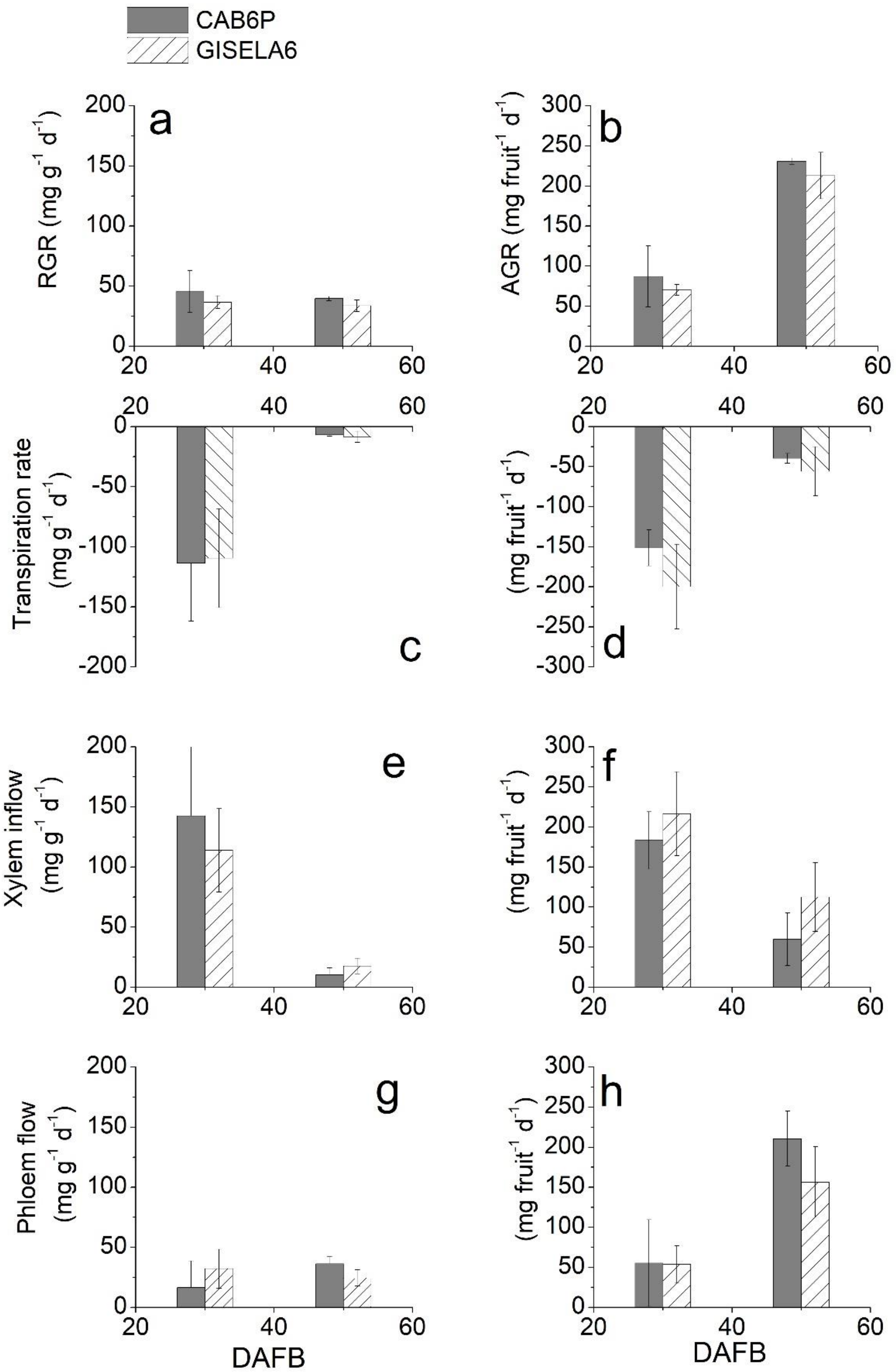

FIGURE 7: Mean (± SE) relative (RGR) (a) and absolute (AGR) (b) growth rates, specific (c) and whole fruit transpiration (d), specific (e) and whole fruit xylem flow (f), specific (g) and whole fruit phloem flow (h) from/to cherry fruit of 'Black Star' sweet cherry trees grafted on CAB6P (grey bars)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

	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. *:$P<0.05$