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

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

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14 SUMMARY

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

33

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

35

36 ABBREVIATIONS:

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

39

40 1. INTRODUCTION

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

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

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

67 Rootstock vigor can also affect productivity and quality of the production (Lang, 2000; Neilsen et al.,
68 2016), due to variations in the hydraulic anatomy and in the water relations of the scion (Peschiutta
69 et al., 2013), although crop load and thus leaf/fruit ratio, seems to be the major driver affecting fruit
70 development and final fruit quality (Whiting and Lang, 2004; Neilsen et al., 2016). Regarding fruit
71 quality, Gonçalves et al. (2006b) showed how thanks to their better water status, vigorous rootstocks
72 usually show a higher fruit size, also because the combination between autofertile cultivars and
73 dwarfing rootstocks may induce excessive crop loads (Bassi 2005). However, despite the wide
74 differences on yield and quality of the production, it is not clear yet how vigor affects the
75 developmental physiology of fruit growth and the evolution of the quality traits within the fruit.

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

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

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

101

102 2. MATERIALS AND METHODS

103 2.1 *Plant material and experimental set up.*

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

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

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

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

123

124 *2.2 Seasonal fruit and shoot growth*

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

128

$$129 \text{ Eq 1: } FW(g) = a * D(mm)^b$$

130

131 where a and b were 0.0021 and 2.5431. This equation was obtained by regressing diameter and weight
132 data of a large number (above 300) of Black Star cherry fruit picked over several seasons, from the
133 same orchard. The R² of the relationship was >0.99.

134

135 *2.3 Water relations*

136 Stem (Ψ_{stem}), leaf (Ψ_{leaf}) and fruit (Ψ_{fruit}) water potentials were monitored at predawn and at 9.00,
137 12.30 and 16.30 hours, on 4 trees per treatment, using a Scholander (Soilmoisture Equipment Corp.
138 Santa Barbara, U.S.A.) pressure chamber. Ψ_{leaf} was measured on one well exposed shoot leaf per tree,
139 on 4 trees per treatment following Turner and Long (1980). Ψ_{stem} was measured on the same trees:
140 one leaf per tree placed in the inner part of the canopy, very close to the main stem, was chosen and
141 covered with aluminium foil at least 90 minutes prior to measurement to allow equilibration with the
142 stem, according to the methodology described by McCutchan and Shackel (1992) and by Naor *et al*
143 (1995). Similarly, Ψ_{fruit} was measured on one fruit per tree, with a total of 4 fruit per treatment.

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

147

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

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

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

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

198

199 Eq. 2: $P_t = N_t - G_t$

200 Eq. 3: $X_t = G_t - D_t$

201 Eq. 4: $T_t = D_t$

202

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

207

208 Eq. 5: $\text{AGR}_{t1} = (\text{FW}_{t1} - \text{FW}_{t0}) / (t1 - t0)$

209

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

212

213 Eq. 6: $\text{RGR}_{t1} = (\text{FW}_{t1} - \text{FW}_{t0}) / (t1 - t0) * \text{FW}_{t0}$

214

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 (Ψ_{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. $0.37 \text{ g fruit}^{-1} \text{ day}^{-1}$ at 50-55 DAFB, in correspondence with fruit
246 cell expansion (Fig. 2b).

247

248 *3.2 Water Relations*

249 In both dates, no difference was recorded in pre-dawn Ψ_{stem} and Ψ_{fruit} between the two rootstocks.
250 However, as time passed by, trees on Gisela™ 6 reached and maintained significantly lower Ψ_{stem}
251 during the day, compared to CAB6P, with minimum values of -0.8 ± 0.01 and -0.4 ± 0.01 MPa at 47
252 DAFB and of -0.86 ± 0.04 and -0.42 ± 0.01 MPa at 55 DAFB, for Gisela™ 6 and CAB6P, respectively
253 (Fig 3a, c). Ψ_{leaf} never showed statistical differences between the two rootstocks, except at 55 DAFB,
254 9:00 hour, when Gisela™ 6 leaves showed slightly lower values compared to CAB6P (Fig 3c). Unlike
255 leaves, Ψ_{fruit} was more affected by the rootstock, with fruit on Gisela™ 6 maintaining lower Ψ_{fruit}
256 values compared to fruit on CAB6P. These differences appeared already at 47 DAFB, becoming more
257 evident at 55 DAFB, with pre-dawn values of about -1.53 ± 0.03 and -1.12 ± 0.05 MPa for Gisela™ 6
258 and CAB6 fruit, respectively (Fig 3b, d).

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

264

265 *3.3 Leaf gas exchanges and water use efficiency*

266 CAB6P leaves showed a higher stomatal conductance at 9:00 and 16:00 hour, with values of
267 0.30 ± 0.03 and 0.22 ± 0.04 mol m⁻² s⁻¹, respectively (Fig 5b). At the same hours, Gisela™ 6 leaves
268 showed a decrease of about 30% and 50%, respectively (Fig 5b). Despite such an important decrease
269 in stomatal conductance, these leaves did not show a significant reduction in photosynthesis, with
270 values of 13.7 ± 0.9 and 11.3 ± 0.8 $\mu\text{mol CO}_2$ m⁻² s⁻¹ at 9:00 and 16:00 hour, respectively (Fig 5a). On
271 the contrary, the daily trend in leaf transpiration followed changes in stomatal conductance with
272 significantly higher water losses from CAB6P leaves, that reached values of about 4.3 ± 0.6 mmol H₂O
273 m⁻² s⁻¹ at 9:00 hour (Fig 5c).

274 At 55 DAFB, the two rootstocks maintained similar photosynthesis and stomatal conductance at 9:00
275 and 12:00 hour, while at 16:30 hour Gisela™ 6 leaves were subjected to a 25% and a 50% reduction
276 compared to CAB6P, respectively (Fig 6a, b). Water losses by transpiration maintained steady values
277 during the day in Gisela™ 6 leaves, while CAB6P showed important increases in leaf transpiration
278 that reached values of 6.4 ± 0.2 mmol H₂O m⁻² s⁻¹, corresponding to almost the double amount of water
279 transpired by Gisela™ 6 leaves, at the same time of day (Fig 6c).

280 Water use efficiency, expressed as the amount of CO₂ fixed per water transpired ($\mu\text{mol CO}_2$ / mmol
281 H₂O) was much higher for Gisela™ 6 both at 47 and 55 DAFB, with values that were ca. 30% higher
282 than CAB6P, regardless of the date and time of day (Fig 5d and 6d).

283

284 *3.4 Fruit growth and vascular flows*

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

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

299 For both rootstocks, the relative contribution of the xylem and phloem flow to fruit growth was about
300 80 and 20% at 30 DAFB and changed to ca. 25 and 75% at 50 DAFB, respectively. Cherry fruit lost
301 70 and 20 % of their daily inflows by epidermis transpiration, at 30 e 50 DAFB, respectively.

302

303 *3.5 Harvest Yield and fruit quality*

304 Harvest data showed average yields of 2.59 and 1.99 kg/tree for trees grafted on Gisela™ 6 and on
305 CAB6P, respectively, while similar average fruit weight (ca. 8 g/fruit) were obtained for the two
306 rootstocks. Despite yield was not significantly different between the two rootstocks, these values
307 indicate a higher crop load on Gisela™6 trees. Soluble solids content was higher for fruit on Gisela™
308 6, with values of 19.5 °Brix, against the 17.18 °Brix recorded from CAB6P fruit (Table 1).

309

310 4. DISCUSSION

311 As reported in literature (Gonçalves et al. 2006a; Ljubojevuc et al. 2013,), rootstock vigor deeply
312 affected the scion vegetative growth, with higher shoot lengths and growth rates on trees grafted on
313 CAB6P, from the beginning of the season, until harvest (Fig. 2a). On the contrary, shoots on the more
314 dwarfing rootstock Gisela™6 grew slowly, with minima in growth velocity in correspondence with
315 the fruit cell expansion stage, the period of maximum fruit growth (Fig. 2a). The slower growth of
316 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 Brüggewirth 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 (Brüggewirth 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

351 values on Gisela™ 6 trees, compared to CAB6P (Fig 4). The lower Ψ_{stem} indicates the difficulty of
352 dwarfing rootstocks to meet their canopy transpiration demand due: i) to the lower absorption
353 capacity of their root system (which is less developed) and ii) to the lower hydraulic conductivity in
354 the grafting point and in the scion (Gonçalves et al. 2005, 2006). As flows of water and carbohydrates
355 move following Ψ gradients within the vascular system (Munch, 1930; Patrick, 1990), the more
356 negative the Ψ_{stem} , the higher will be the need for shoots and fruit to decrease their Ψ in order to
357 attract water and carbohydrate resources towards themselves. Despite the reduction in Ψ_{stem} , Gisela™
358 6 leaves maintained similar Ψ_{leaf} to CAB6P (Fig. 3), resulting in lower stem-to-leaf Ψ gradients (Fig
359 4) during most of the day and thus in a lower capacity to attract water. The same mechanisms can
360 apply to shoot tips, which might then lack the necessary turgor for their growth.

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

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

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

393

394 CONCLUSIONS

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

- 398 i) When water is non-limiting, trees on dwarfing rootstocks maintain enough photosynthetic
399 capacity to fully sustain their fruit growth. This occurs thanks to stomatal conductance
400 values that, although reduced in comparison with more vigorous rootstocks, are still not
401 limiting for photosynthesis.
- 402 ii) Changes in whole canopy water relations decrease the sink strength of shoots and lead to
403 a shift in the relative partitioning of water and carbohydrates towards fruit sinks. This
404 seems to occur thanks to an adaptation capacity of fruit that, unlike leaves, are more able
405 to lower their Ψ_{fruit} to keep up with the lower Ψ_{stem} typical of dwarfing rootstocks. On the
406 other hand, high-vigor rootstocks appear to physiologically shift towards higher
407 vegetative sink strength thanks to the relatively higher Ψ gradient between stem and
408 leaves.

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

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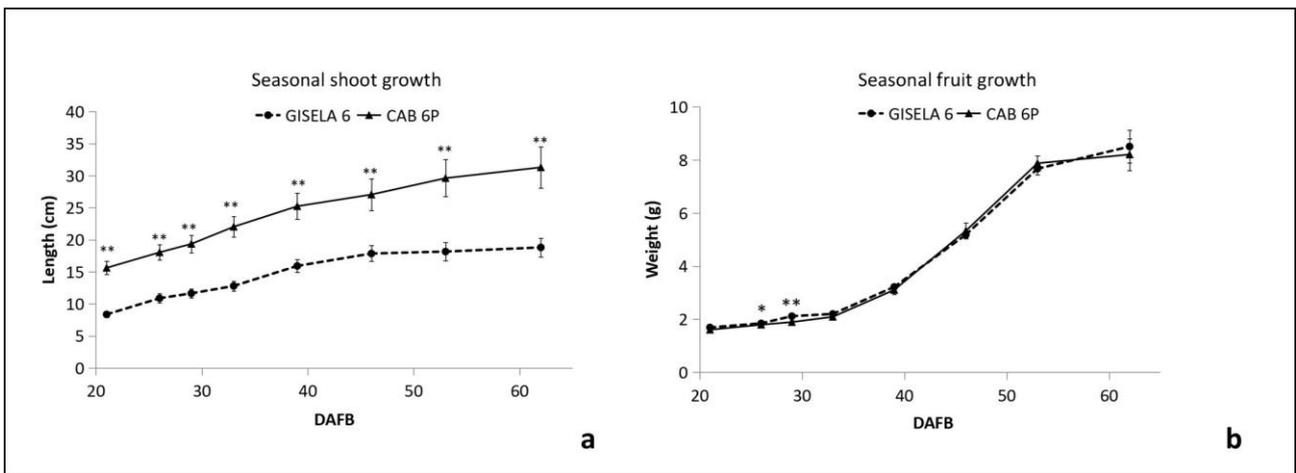
545 This research did not receive any specific grant from funding agencies in the public, commercial, or
546 not-for-profit sectors.

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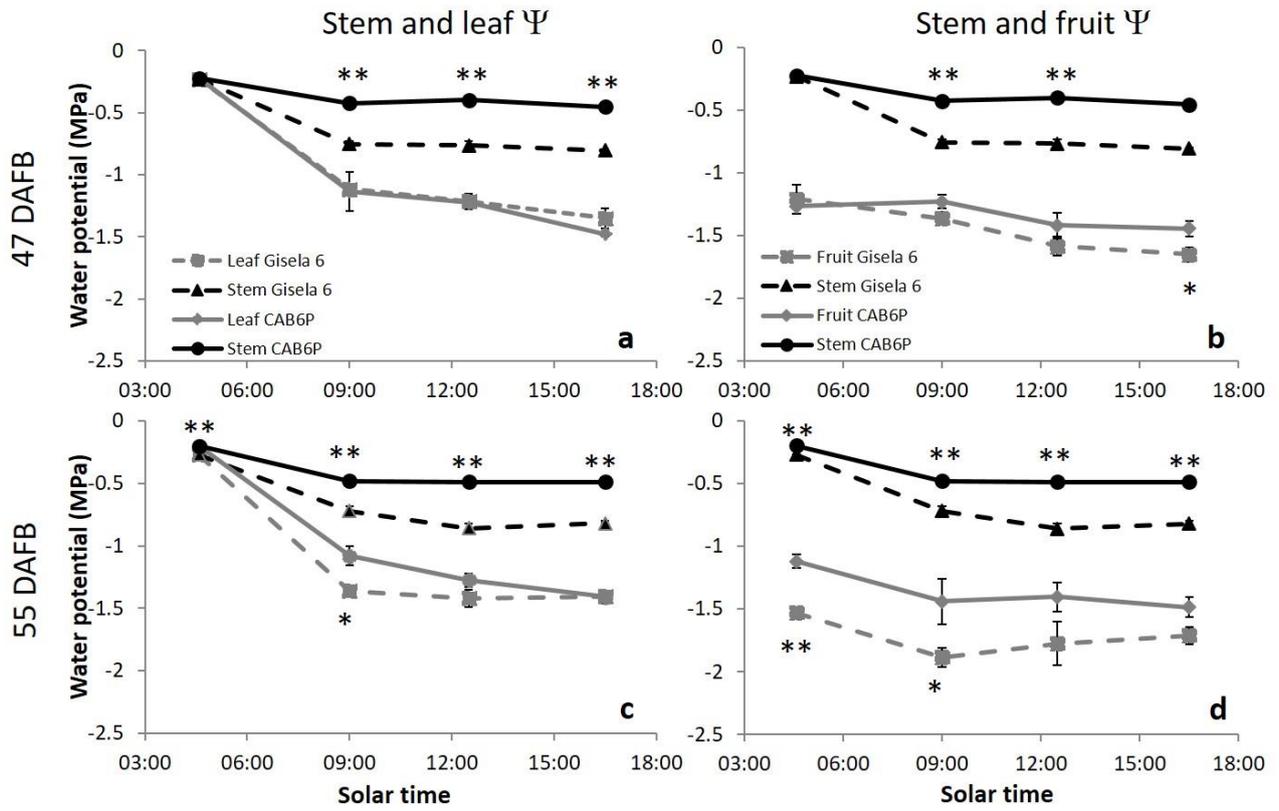
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 551 FIGURE 1 Gauge for the continuous monitoring of fruit growth of ‘Black Star’ sweet cherry at 55
 552 DAFB.
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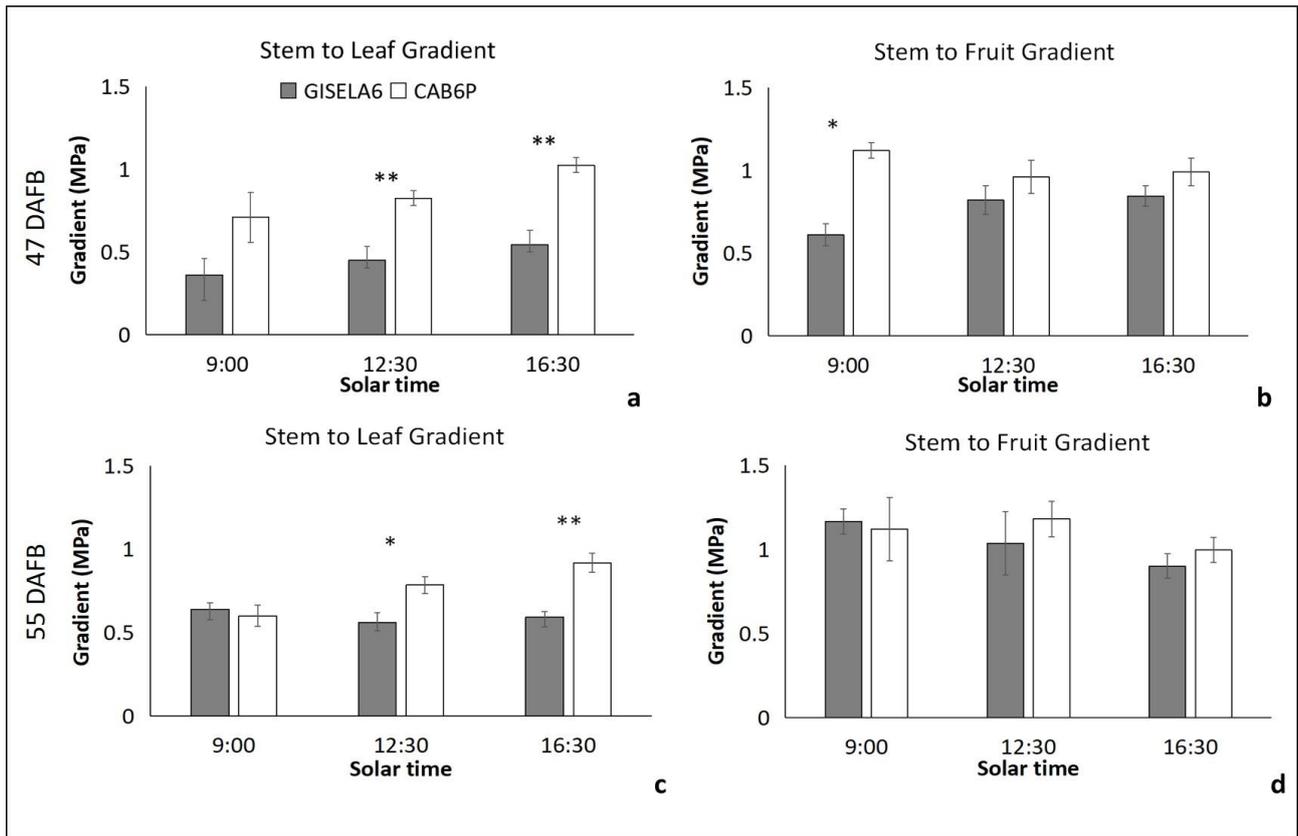


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 555 FIGURE 2: Seasonal shoot (a) and fruit (b) growth pattern of ‘Black Star’ sweet cherry trees grafted
 556 on CAB6P (continuous line) and Gisela™ 6 (dashed line) rootstocks. Each point represents the mean
 557 (\pm SE) of 48 shoots and 64 fruit, respectively. Statistical comparison between rootstocks with
 558 Student’s t test. *:P<0.05 **:P<0.01
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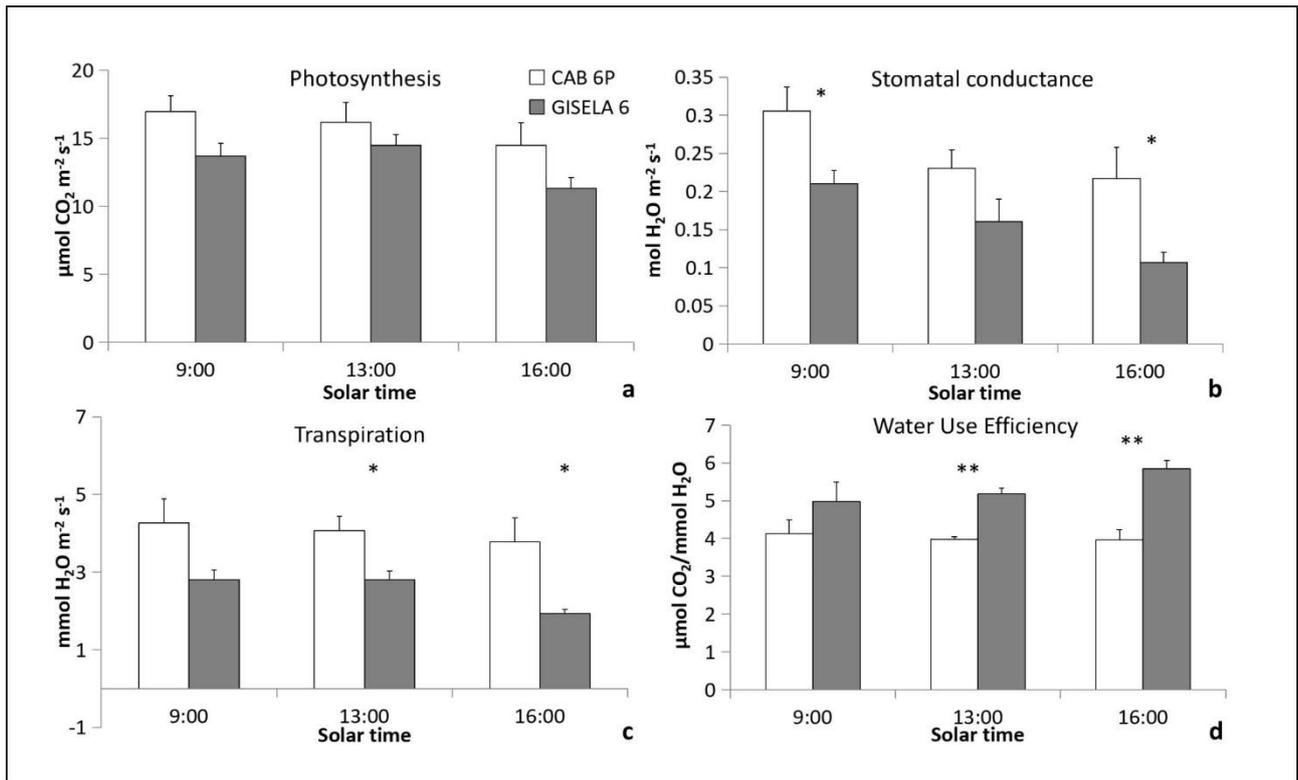
563 FIGURE 3 Diurnal pattern of stem (black lines) and leaf (grey lines) (a, c) and of stem (black lines)
 564 and fruit (grey lines) water potentials (b, d) measured on ‘Black Star’ sweet cherry trees grafted on
 565 CAB6P (continuous lines) and on Gisela™6 (dashed lines), at 47 (a, b) and at 55 (c, 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 (\pm SE) of 4 replicates.
 572 Statistical comparison between rootstocks with Student's t test. *:P<0.05 **:P<0.01

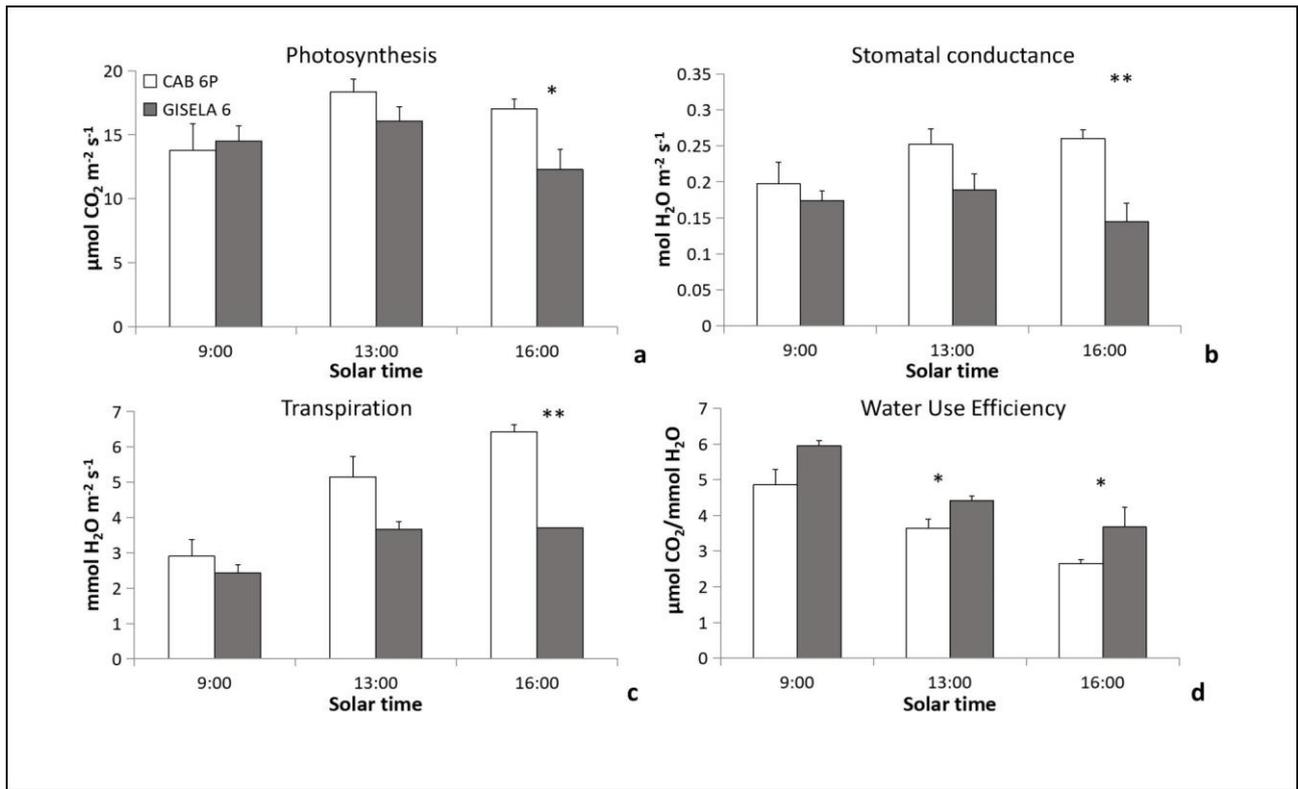
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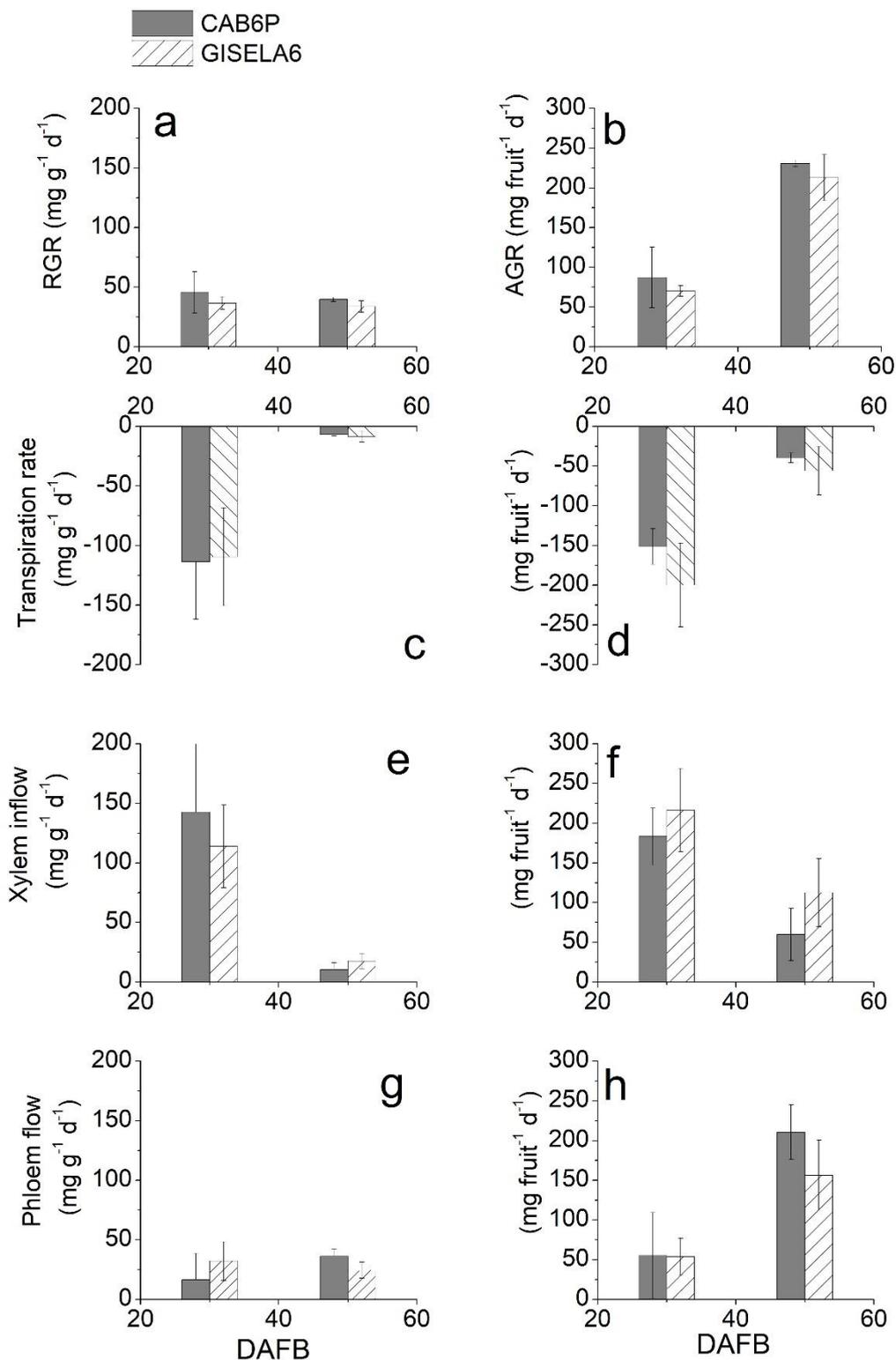
575 FIGURE 5: Leaf net assimilation rate (a), stomatal conductance (b), leaf transpiration (c) and water
 576 use efficiency (WUE) recorded at 9:00, 13:00 and 16:00 hour, at 47 DAFB for ‘Black Star’ sweet
 577 cherry trees on CAB6P (grey bars) and Gisela™ 6 (white bars) rootstocks. Each bar reports the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

580



581

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



587

588 FIGURE 7: Mean (\pm 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	

594

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

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599