

Research paper

Effect of tree microenvironment on sweet cherry fruit transpiration, xylem, and phloem flows

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ABSTRACT

This study provides an overview of how shading covers can affect air vapor pressure deficit (VPD) and sweet cherry water status, and its fruit absolute growth rate (AGR), and its xylem (X), phloem (P), and transpiration (T) flows. The experiment was carried out from April to June 2024 on a 14-year-old sweet cherry orchard, variety 'Sweet Saretta' grafted on 'Gisela 6'. These trees were cultivated under: (i) direct sunlight conditions, and (ii) a 20 % shading black net. During the experiment, environmental conditions, such as air temperature (T_a) and relative humidity (RH), were monitored, and from this data the VPD was calculated. Tree water status was assessed by measuring at 9:00 h stomatal conductance (g_s), and net photosynthesis (P_n) and at 12:00 h stem water potential (Ψ_{stem}). During the experiment, the fruit equatorial diameter was measured at regular time intervals during the season. At the same time, fruit daily diameter variations were monitored precisely every 30 min at 33, 38, and 43 days after full bloom, corresponding with the early and late stage II, and with the early stage III of fruit growth. From these data, the 30-minute fruit AGR, X, P, and T flows were estimated, and their daily contribution was calculated. In addition, fruit X, P, and T flows were correlated with VPD at different fruit growth stages. During the experiment, VPD was reduced on average by 19 % under the net. At the same time, trees cultivated under a net showed less negative values of Ψ_{stem} and higher values of g_s and P_n . Differences between treatments were also observed in the 30-minute and daily contributions of fruit AGR, X, P, and T flows. Results showed that cropping condition slightly affected the relationship between VPD and fruit T, X, and P flow. In conclusion, under netting, the lower air VPD had some positive effects on tree water status and seasonal fruit growth. These conditions mainly minimized the daily contribution of fruit T, X, and P flows, except in the case of P flow at stage III, and did not significantly affect the correlation between these flows and the VPD.

1. Introduction

Currently, agricultural production is under threat due to the negative impact of global warming and water scarcity on fruit growth. Nets could mitigate these undesirable effects by protecting crops from both abiotic and biotic stresses, such as pests or diseases (Manja and Aoun, 2019). The nets can be characterized by different materials, colours, shading percentages, and functions, i.e., anti-rain, anti-hail, and anti-insect. Depending on the material and colour of the net, modifications in the air temperature (Germanà et al., 2003; Incesu et al., 2016; Overbeck et al., 2018), relative humidity (Blakey et al., 2016; Tinyane et al., 2018) and incoming solar radiation (García-Sánchez et al., 2015; Mira-García et al., 2020) have been reported. Additionally, covers could also affect

tree water status and, more concretely, leaf gas exchange and stem water potential, and also the photosystem efficiency (Cohen et al., 2005; Incesu et al., 2016; Jifon and Syvertsen, 2003; Lopez et al., 2018; Shafiq et al., 2020; Williams, 2012). These modifications to the tree's water status observed under netting can alter its water potential gradients, and fruit growth that is governed by the balance among xylem (X), phloem (P), and transpiration (T) in/out flows (Giovannini et al., 2022; Morandi et al., 2007).

In a fruit, the X flow transports water, minerals and metabolites, whereas the P flow translocates water, minerals and photoassimilates from the source to the sink organs (Braun, 2024; De Boer and Volkov, 2003; Peuke, 2010). In trees, both flows are mainly driven passively, with no energy requirement; X responds to water potential gradients in

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the xylem vessels (White and Ding, 2023), while P responds to turgor pressure gradients (Münch, 1930). At the fruit level, phloem unloading can happen both passively (through the symplast) and/or thanks to an active transport called ‘apoplastic’, which drives carbohydrates from the P to the fruit parenchymatic cells against concentration and/or pressure gradients. This step requires the presence of specific carbohydrate transporters (Braun, 2024; Patrick, 1997). In contrast to X and P flows, T refers to water that is lost to the atmosphere via the fruit epidermis. This process is always passively driven in the fruits and is a function of vapor pressure deficit (VPD), fruit surface conductance, and skin speckle (Gibert et al., 2005; Rossi et al., 2022; Wu et al., 2003).

As these flows govern fruit growth, studying the effect of the net on them is essential, especially on sweet cherry, a crop in which covers are commonly used. However, to our knowledge, no scientific information exists on the effect of net on sweet cherry eco physiologic performance. The contribution of fruit X, P, and T flows to sweet cherry development is well known, with xylem and phloem decreasing and increasing their contribution during fruit development, respectively (Brüggenwirth et al., 2016). However, the response of fruit vascular flows under netting has never been studied. At the same time, the nets can also modify the tree microenvironment, which in turn, can affect fruit X, P, and T flows and finally its growth. However, the effect of this cropping system on the relationship between microclimate and these flows was not deeply studied on this crop. In this sense, this study provides an overview of how nets affect the tree microenvironment and water status, as well as the fruit absolute growth rate (AGR) and its X, P, and T flows. It also includes complete information regarding the effect of the net on the relationship between fruit X, P, and T flows and VPD along sweet cherry development. The experimental hypothesis is that net can reduce the VPD, having a positive effect on tree water status, and fruit water inputs via X, P, and outputs via T, and therefore it affects AGR.

2. Materials and methods

2.1. Experimental conditions

The trial was conducted from April to June 2024 in a 1-ha sweet cherry orchard in Cadriano, Bologna, Italy (44°32'54.5"N 11°23'13.2"E). The experimental plot was cultivated with 14-year-old ‘Sweet Saretta’ trees, grafted on ‘Gisela 6’ rootstock, planted at 4 × 1 m. The irrigation system consisted of a simple drip line with two pressure-compensated emitters of 4 L h⁻¹ per tree. The following treatments were compared: (i) ‘control’, in which the trees were cultivated under direct sunlight, and (ii) ‘net’, in which the trees were cultivated under a black polyethylene net, 20 % shading. The net was opened on 15 April and closed on 30 June 2024. Each cropping condition consisted of a subplot of 24 trees, 4 of which were selected for experimental measurements; the others were considered as borders. Both systems were irrigated equally and in accordance with the IRRIFRAME (<https://www.irriframe.it/Irriframe>) recommendations, to meet the trees’ evapotranspiration requirements.

2.2. Measurements

2.2.1. Meteorological conditions

During the experiment, air temperature (T_a) and relative humidity (RH) were continuously monitored with combined T/RH sensors (model SHT35, company Sensirion, Stäfa, Switzerland), one in each cropping condition. These sensors were connected to a wireless node system (model NOD-AQ, company Winet, Cesena, Italy), reading values every 5 min and recording average values every 30 min. The system sent the data to a gateway connected to Winet’s cloud-based web server platform, <https://winetsrl.cloud/v2/index.php> (company Winet, Cesena, Italy), for data processing and visualization. The vapor pressure deficit (VPD) was calculated from T and RH data, according to Allen et al. (1998).

2.2.2. Tree water status

To assess tree water status, stem water potential (Ψ_{stem}), stomatal conductance (g_s), and net photosynthesis (P_n) were measured approximately every 15 days from April to June 2024, on one leaf per experimental tree, with a total of four leaves per treatment. The Ψ_{stem} was measured at 12:00 h solar time, using a Scholander pressure chamber (model 3000, company Soil Moisture, California, United States) according to the recommendations of McCutchan and Shackel, (1992). Leaves on the inner part of the canopy were selected and covered with aluminum foil at least 1,5 h before the measurements. On the same days, g_s and P_n were measured at 9:00 h solar time on sunny leaves using a portable photosynthesis system (model LI-6800, company LI-COR, Nebraska, United States) equipped with a leaf chamber of 2 cm². During the measurement, the light intensity, CO₂ concentration, and air flow rate in the chamber were 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 400 $\mu\text{mol mol}^{-1}$ and 450 $\mu\text{mol s}^{-1}$, respectively.

2.2.3. Seasonal fruit growth

At 23, 31, 38, 44, 52, and 59 days after full bloom (DAFB), the equatorial diameter of six fruits per experimental tree, three on each side of the canopy, was measured with a digital caliper (model CD-15D, company Mitutoyo, Sakado, Japan). After that, the equatorial diameter (D) of each fruit in mm was converted to fresh weight (FW) in g using the following equation:

$$\text{FW} = (a \cdot D)^b \quad (1)$$

Where a and b were 0.0017 and 2.6166. This equation was derived by regressing equatorial diameter and fresh weight data of a large number (over 300) of ‘Sweet Saretta’ sweet cherry fruits picked over several seasons, from the same orchard. The Pearson’s coefficient (r) of the relationship was greater than 0.99. In this study, the fruit started changing colour after May 15th 2024, 43 days after full bloom (DAFB) and the harvest data was June 7th 2024, 66 DAFB.

2.2.4. Fruit absolute growth rate and transpiration, phloem, and xylem flows

At 33, 38, and 43 DAFB, coinciding with the fruit phenological stage of early and late stage II and early stage III, respectively, equatorial diameter variations were monitored on three fruits per experimental tree, 12 per treatment. On these fruits, diameter variations were monitored with custom-built fruit gauges (Morandi et al., 2007), consisting of a light, stainless steel frame supporting an electronic sensor (Megatron Elektronik AG & Co., Munich, Germany). These sensors were connected to a wireless data logger system, which read values every 5 min and recorded average values every 30 min. The system sent the data to a gateway connected to Winet’s cloud-based web server platform, <https://winetsrl.cloud/v2/index.php> (company Winet, Cesena, Italy), for data processing and visualization. Of the three fruits per tree in which equatorial diameter variations were measured, one was kept intact (I) (no changes made); another was girdled (G), in which the phloem tissue was removed with a pocket knife, leaving only the xylem tissue; and the other was detached (D), where all vascular tissues were removed from the tree. During the experimental period, G and D fruit were replaced every 48 h, whereas I fruit remained the same during the experiment. Intact, Girdled and Detached fruit were always selected with similar size. Equatorial diameter variations of G, D, and I fruits were used to calculate their transpiration (T), xylem (X), and phloem (P) flows according to Lang (1990). This approach assumes that: (i) fruit equatorial diameter variations in a given time interval are the result of the algebraic sum among P, X, and T, (ii) X flow is not affected by girdling, and (iii) T rate is not affected by detachment. Taking into account these assumptions, fruit T, X, and P flows in a given time interval (t) were calculated as:

$$T_t = D_t \quad (2)$$

$$X_t = I_t - D_t \quad (3)$$

$$P_t = I_t - G_t \quad (4)$$

Moreover, on the same days, the fruit absolute growth rate (AGR) was calculated as:

$$AGR = P_t + X_t - T_t \quad (5)$$

From these data, the daily contribution of fruit AGR and its T, X, and P flows were calculated as the cumulative sum of the 30-minute data per day. Also, the effect of net on the relationships between the VPD and fruit X, P, and T flows was studied in detail. For this purpose, on each date, 30-minute data of VPD and fruit X, P, and T flows were correlated.

2.2.5. Statistical analysis

A student's *t*-test ($p < 0.05$) was performed to assess the statistical difference between treatments in the Ψ_{stem} , g_s , P_n , and seasonal fruit growth. As the data did not follow a normal distribution, a Mann-Whitney *U* test ($p < 0.05$) was carried out to assess statistical differences between treatments on the 30-minute and daily cumulative sum of fruit AGR, and its X, P, and T flows. Linear and quadratic regression analyses were performed to obtain Pearson's coefficient (*r*) and the significance of the relationships between VPD and 30-minute data of fruit T, X, and P flows. Moreover, test-*t* was carried out to assess the statistical difference between treatments on the slope and the intercept of these regressions. The statistical analysis was carried out using the SPSS statistical software (version 25, International Business Machines (IBM), New York, USA)

3. Results

3.1. Meteorological conditions

During the experimental period, the average daily air vapor pressure deficit (VPD) fluctuated wildly from day to day (Fig. 1). The highest values were measured at the end of the experiment, specifically at 40 DAFB, being 0.9 and 0.7 kPa in the control and net treatments, respectively. In contrast, the lowest values were observed at 35 DAFB, at the beginning of the experiment, and were below 0.2 kPa in both treatments. These low VPD values were due to the low average daily T_a and high relative humidity (RH) on both treatments, with values around 14°C and 90 %, respectively (data not shown). During the experiment, the VPD was usually higher in the control treatment than under the net (Fig. 1) where it was reduced by an average of 19 %.

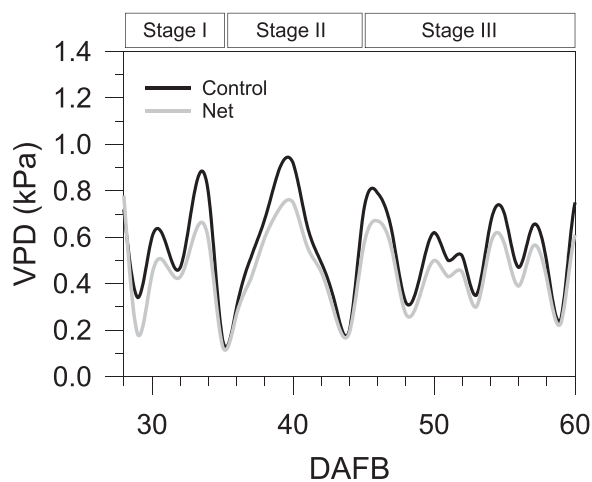


Fig. 1. Seasonal trend of the average daily vapor pressure deficit (VPD) in the control and net treatments at the phenological stages of fruit growth I, II, and III. DAFB means days after full bloom.

3.2. Tree water status

In this study, stem water potential (Ψ_{stem}) gradually decreased from the early stage II to the early stage III of fruit growth. Ψ_{stem} was always more negative in trees growing under control conditions, where it ranged from -0.7 to -1.0 MPa. Whereas in the net treatment it ranged from -0.5 to -0.6 MPa (Fig. 2A). Difference between the two treatments was highest in the early stage III, with Ψ_{stem} being 38 % higher (less negative) under netting. Tree net photosynthesis (P_n) was the highest at the early fruit growth stage III. In general, trees grown in control conditions showed the lowest P_n values, ranging from 11.1 to 16.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas in the net treatment, it ranged from 15.7 to 22.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2B). Difference between the two treatments was maximum at the early stage III, with P_n being 34 % higher under the net. Stomatal conductance (g_s) was the lower in control trees, ranging from 272.1 to 277.7 $\text{mmol m}^{-2} \text{s}^{-1}$, while under netting it ranged from 378.6 to 504.8 $\text{mmol m}^{-2} \text{s}^{-1}$ (Fig. 2C). The difference between the two treatments was most significant at the early stage III, being 60 % higher in the net treatment than in the control treatment.

3.3. Seasonal fruit growth

Fruit growth increased progressively from 23 to 59 DAFB in both treatments. In general, fruit fresh weight maintained higher values

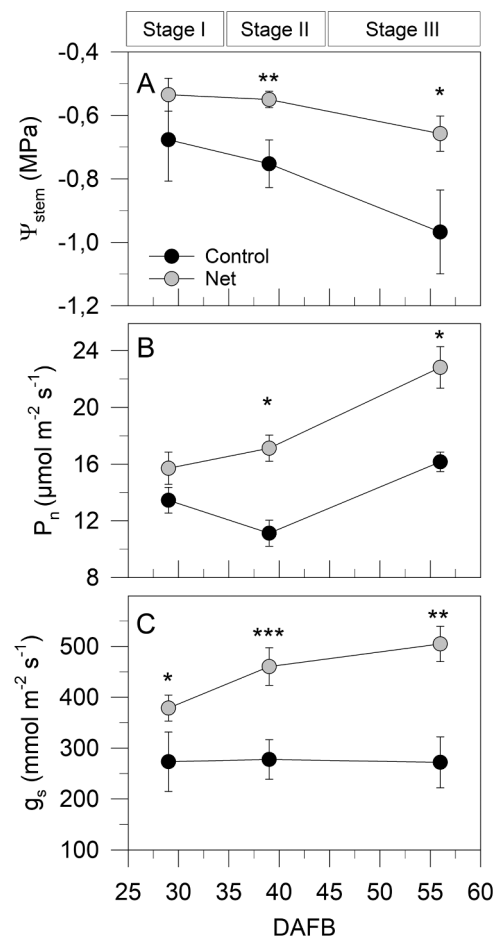


Fig. 2. Seasonal pattern of stem water potential (Ψ_{stem}) (A), net photosynthesis (P_n) (B), and stomatal conductance (g_s) (C) in control and netted sweet cherry trees at the phenological stages of fruit growth I, II, and III. Each point is the average of 4 replicates. Vertical bars on the data points are \pm standard errors. DAFB means days after full bloom. *, **, and *** indicate statistically significant differences between treatments according to Student's *t*-test at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

under the net treatment, ranging from 2.0 to 12.3 g (Fig. 3), while in the control treatment, it showed values ranging from 1.7 to 9.8 g. Differences between treatments were observed from 38 DAFB onwards (Fig. 3). The maximum difference in the fruit growth between treatments was observed at 52 DAFB. On this date, netted fruits weight was on average 2.7 g higher than the control ones (Fig. 3).

3.4. Daily course of 30-minute data of vapor pressure deficit, fruit absolute growth rate, and its transpiration, xylem, and phloem flows

In both treatments, VPD showed the highest values in the central hours of the day and the lowest in the early morning and at night (Figs. 4–6A). Daily mean VPD values differed slightly between the two treatments, decreasing under the net by 7 %, 9 %, and 15 % in the early and late stage II, and in the early stage III of fruit growth, respectively.

Fruit T flow followed VPD, with the lowest (more negative) values in the central hours of the day and the highest (less negative) values in the early morning and evening on both treatments (Figs. 4–6B). In the control treatment, it ranged from 0 to $-0.7 \text{ mg fruit}^{-1} \text{ min}^{-1}$, from -0.0 to $-1.1 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and from -0.1 to $-0.8 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stage II, and early stage III of fruit growth, respectively (Figs. 4–6B). Under the net, it ranged from 0 to $-0.7 \text{ mg fruit}^{-1} \text{ min}^{-1}$, from -0.0 to $-0.9 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and from -0.1 to $-0.6 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stage II, and early stage III of fruit growth, respectively. During the central hours of the day, from 12:00–16:00 h, fruit T flow was most negative in the control treatment (Figs. 4–6B). Its daily average was 5 %, 20 %, and 21 % higher in the control treatment than under net at early and late stage II, and early stage III of fruit growth, respectively.

Fruit X flow increased progressively from 08:00–20:00 h, with the highest values in the late afternoon and the lowest in the early morning and night hours (Figs. 4–6C). In the control treatment, it ranged between 0.1 and $0.6 \text{ mg fruit}^{-1} \text{ min}^{-1}$, from 0.2 to $1.12 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and from 0.1 to $0.6 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stages II, and at early stage III of fruit growth, respectively. In the net treatment, it ranged from 0.0 to $0.6 \text{ mg fruit}^{-1} \text{ min}^{-1}$, 0.1 – $0.9 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and 0.0 – $0.5 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stage II, and at early stage III of fruit growth, respectively (Figs. 4–6C). In the afternoon, fruit X flow was significantly higher in the control than in the net treatment. Indeed, its daily average was 16 %, 16 % and 13 % higher in the control than in the net treatment, at early and late stage II, and at early stage III of fruit growth, respectively (Figs. 4–6C).

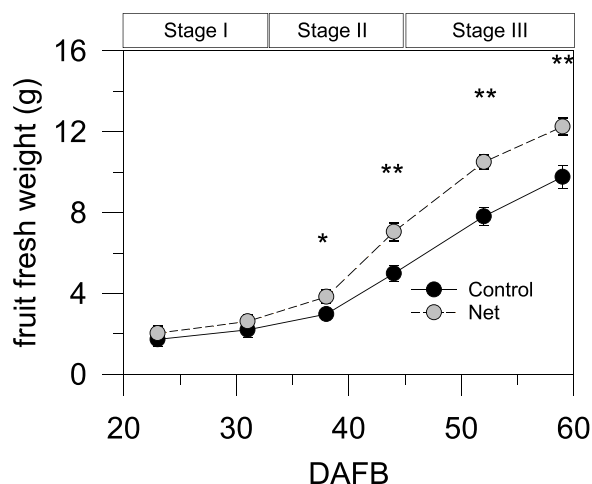


Fig. 3. Seasonal fruit growth in control and netted sweet cherry trees at the phenological stages of fruit growth I, II, and III. Each point represents the mean \pm standard error of 24 fruits. DAFB means days after full bloom. *, and ** indicate statistically significant differences between treatments according to Student's *t*-test at $p < 0.05$ and $p < 0.01$, respectively.

At early and late stage II, the daily course of fruit P flow was completely independent from VPD, varying wildly from hour to hour (Figs. 4–6D). However, at early stage III its daily dynamic coincided with VPD, with the highest values occurring in the central hours of the day and the lowest in the early morning and late afternoon. In the control treatment, fruit P flow ranged from -0.1 – $0.3 \text{ mg fruit}^{-1} \text{ min}^{-1}$, from -0.2 – $0.2 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and from 0.1 to $0.5 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stage II, and at early stage III, respectively. Whereas, in the net treatment, it ranged from -0.1 – $0.3 \text{ mg fruit}^{-1} \text{ min}^{-1}$, from -0.2 – $0.2 \text{ mg fruit}^{-1} \text{ min}^{-1}$, and from 0.2 to $0.7 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stages II, and at early stage III of fruit growth, respectively (Figs. 4–6D). At early and late stage II, the average daily fruit P flow was 20 % and 28 % higher in the control treatment. On the contrary, at early stage III of fruit growth, it was 15 % higher in the net treatment.

Fruit absolute growth rate (AGR) showed a circadian cycle resulting from the balance among T, P, and X flows and showing periods of dehydration and rehydration during the day (Figs. 4–6E). The highest fruit growth rate values were recorded in the early morning, in the late afternoon, and at night. Meanwhile, the lowest fruit AGR was observed from 8:00–18:00 h, coinciding with the highest VPDs (Figs. 4–6A). In both treatments, AGR increased progressively from early stage II to III, where average fruit AGR in the control treatment was almost triple that of the first one, reaching the daily average value of $0.3 \text{ mg fruit}^{-1} \text{ min}^{-1}$. A similar trend was observed in the net treatment, with average values of 0.1, 0.1, and $0.4 \text{ mg fruit}^{-1} \text{ min}^{-1}$ at early and late stage II, and at early stage III of fruit growth, respectively (Figs. 4–6E). At early stage II of fruit growth, some significant differences were observed between treatments (Fig. 4E). However, in late stage II, fruit AGR was, on average, 22 % higher in the control treatment than under net (Fig. 5E). Similarly, in early stage III, fruit AGR was on average 23 % higher under netting (Fig. 6E).

3.5. Relationship between vapor pressure deficit and fruit transpiration, xylem and phloem flows

Regardless of treatment, fruit T strongly correlated with VPD at the different DAFB, with the Pearson's coefficient (*r*) varying from -0.92 to -0.97 (Fig. 7). All relationships were significant at $p < 0.001$. In the control treatment, the highest *r* was recorded in the late stage II of fruit growth, whereas in the net treatment, it was recorded at the end of the experimental period, at early stage III (Figs. 7B and 7C). In the different relationships between VPD and fruit T flow, the *r* was very similar in the two treatments. However, it was slightly higher in the control than in the net treatment, except at early stage III of fruit growth. The correlation between fruit T flow and VPD was negative, or also called inverse, as denoted by the sign of the slope of the linear regression. This means that an increase in the VPD promoted an increase in the fruit T flow, with the latter showing more negative values. During the experiment, the slope of these correlations was higher in the control treatment during early and late stage II of fruit growth, and in the net treatment during early stage III (Fig. 7), even though non-significant differences were observed between treatments. Indeed, non-significant differences were observed between treatments on the intercept of the correlation.

The correlations between fruit X flow and VPD were positive, or also called direct, as denoted by the sign of the slope of the linear regression. All the correlations were significant at $p < 0.001$ (Fig. 8). In the different relationships, *r* was the highest in the control treatment. Regarding the slope of the linear regressions, it was higher in the control than in the net treatment, with even non-significant differences observed between the treatments. In all correlations, the VPD and fruit X flow data were linearly fitted, except in the case of the control treatment at the early stage III of fruit growth (Fig. 8C). At the same time, significant differences between treatments on the intercept of the correlations were only observed during late stage II of fruit growth (Fig. 8B).

Fruit P flow was correlated with VPD only at early stage III of fruit growth, with an *r* of 0.85 and 0.79 in the control and in the net

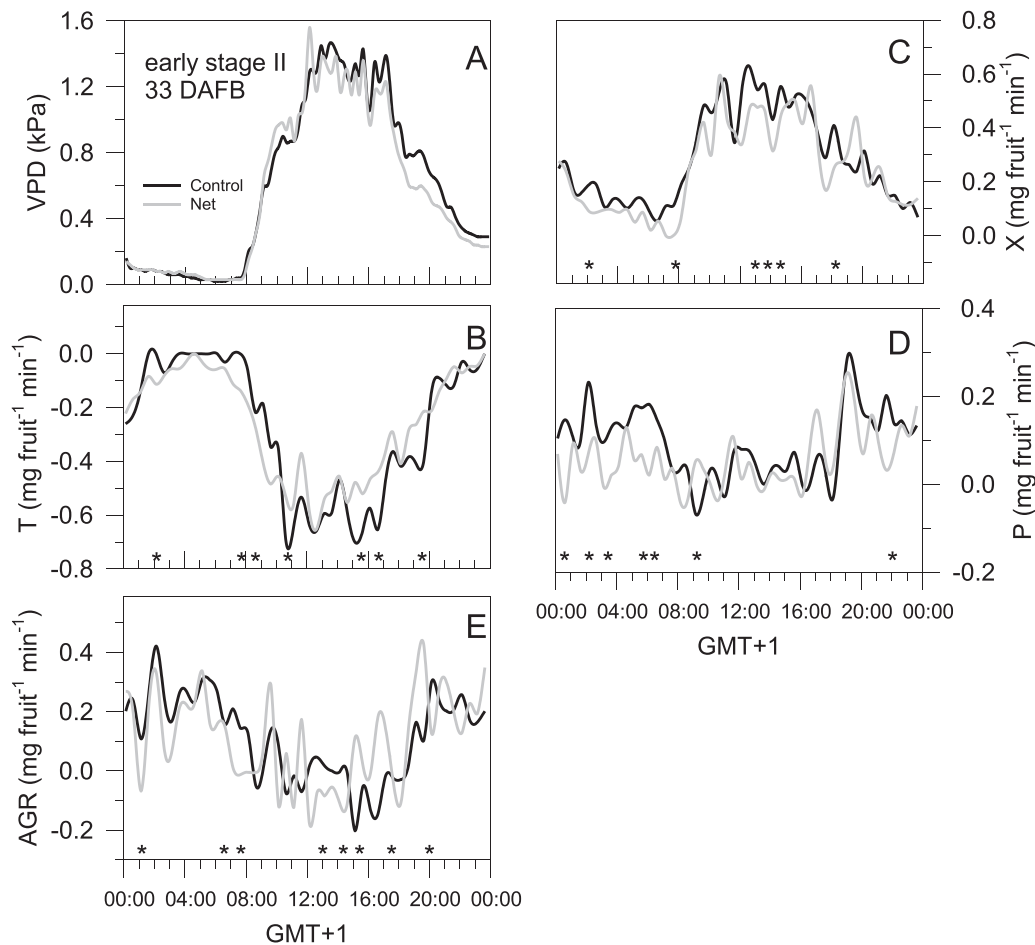


Fig. 4. Daily courses of vapor pressure deficit (VPD) (A), fruit transpiration (T) (B), xylem (X) (C), phloem (P) (D) in/outflows, and absolute growth rate (AGR) (E), at early stage II, in control and netted treatments. Data represent the 30-minute average values per treatment ($n = 4$). * indicates statistically significant differences between treatments according to the Mann-Whitney U test at $p < 0.05$. DAFB means days after full bloom.

treatment, respectively (Fig. 9C). This correlation was positive, meaning that an increase in the VPD promoted a rise in fruit P flow. Significant differences between treatments were observed in the intercept of the correlations at early stage III of fruit growth (Fig. 9C).

3.6. Daily cumulative sum of fruit absolute growth rate, and its transpiration, xylem, and phloem flows

The daily contribution of fruit AGR was very similar between the two treatments at the different fruit growth stages studied (Fig. 10A). At the same time, it increased progressively from early stage II to early stage III in both treatments. In fact, it started with an average of $80.0 \text{ mg fruit}^{-1} \text{ day}^{-1}$ during early stage II of fruit growth in both treatments and reached the values of 214.5 and $271.1 \text{ mg fruit}^{-1} \text{ day}^{-1}$ in the control and in the net treatment, respectively, at early stage III of fruit growth (Fig. 10A).

During the season, the daily contribution of fruit T flow decreased from early stage II to early stage III of fruit growth, reaching the minimum value of -338.0 and $-308 \text{ mg fruit}^{-1} \text{ day}^{-1}$ in the control and in the net treatment, respectively (Fig. 10B). Concretely, during early stage III of fruit growth, it decreased by 46 % and 38 % in the control and net treatment, respectively. Significant differences in the daily contribution of fruit T flow were observed between treatments at late stage II and at early stage III of fruit growth, but not during early stage II, where it was approximately $-180.0 \text{ mg fruit}^{-1} \text{ day}^{-1}$ in both treatments.

The daily contribution of fruit X flow increased progressively from

early to late stage II of fruit growth, reaching the values of 429.5 and $368.2 \text{ mg fruit}^{-1} \text{ day}^{-1}$ in the control and in the net treatment, respectively (Fig. 10C). Subsequently, it decreased from late stage II to early stage III of fruit growth by 42 % and 41 % in the control and net treatment, respectively.

In both treatments, the daily contribution of fruit P flow increased progressively during the season, reaching the maximum values at early stage III of fruit growth (Fig. 10D). From early stage II to early III of fruit growth, it increased until 125.7 and $215.4 \text{ mg fruit}^{-1} \text{ day}^{-1}$ in the control and net treatment, respectively. Significant differences between treatments were only observed at late stage II of fruit growth, with control fruit showing higher P inflows.

4. Discussion

4.1. Effect of nets on canopy microenvironment and tree water status

Nets can induce modifications in the canopy microenvironment. In our study, nets have the capacity to reduce incoming photosynthetically active radiation (PAR) (data not shown), in agreement with results reported previously by other authors (García-Sánchez et al., 2015; Salvadores and Bastías, 2023). This reduction in the PAR observed under netting favoured a decrease in air temperature (T_a) and an increase in its relative humidity (RH) (Blakey et al., 2016; Tinyane et al., 2018; Wachsmann et al., 2014), reducing air vapor pressure deficit (VPD) (Fig. 1), thus creating an optimal microenvironment for crop development. This reduction on the incoming PAR did not affect negatively net

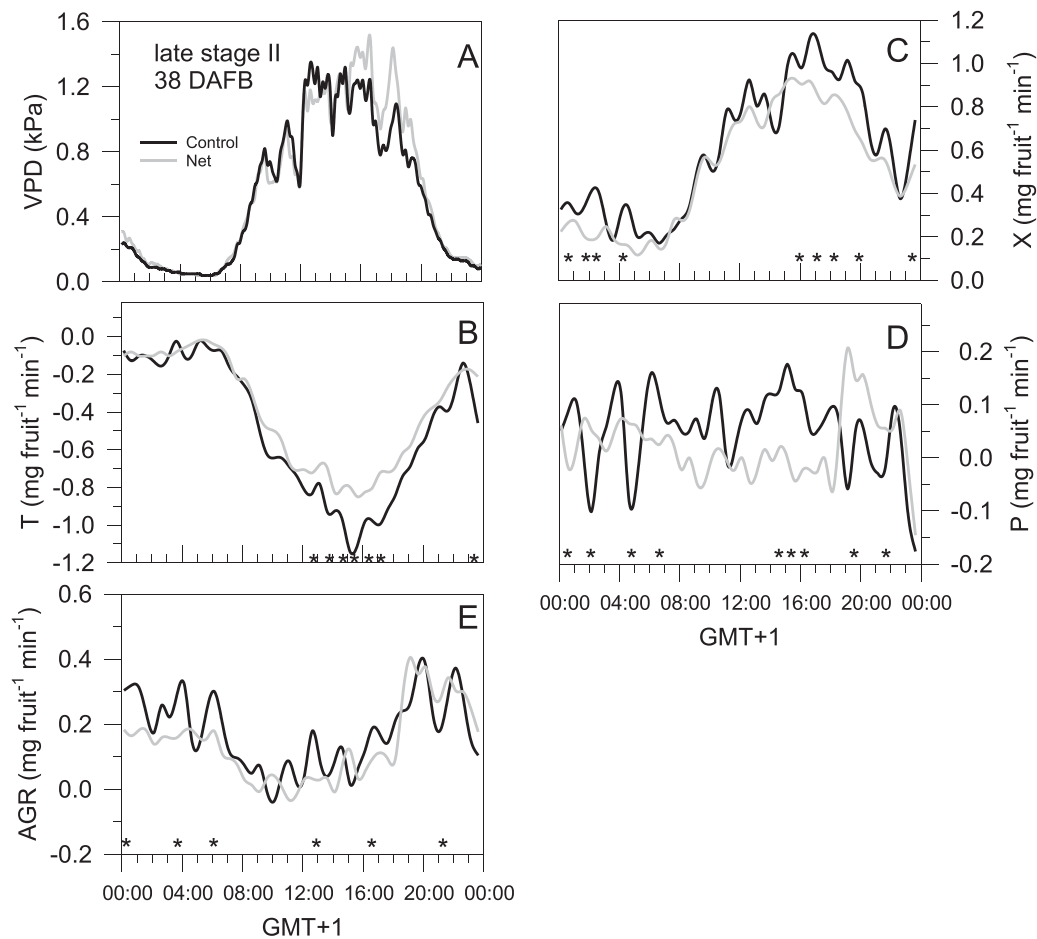


Fig. 5. Daily courses of vapor pressure deficit (VPD) (A), fruit transpiration (T) (B), xylem (X) (C), phloem (P) (D) in/outflows, and absolute growth rate (AGR) (E), at late stage II, in control and netted treatments. Data represent the 30-minute average values per treatment ($n = 4$). * indicate statistically significant differences between treatments according to the Mann-Whitney U test at $p < 0.05$. DAFB means days after full bloom.

photosynthesis (P_n), as demonstrated by the high P_n values observed in trees grown under netting (Fig. 2B). This lack of effect on the photosynthetic performance might be due to the following reasons: (i) the net shading percentage was quite low (20 % only) with radiation reaching the canopy still higher than the saturation point, and (ii) the rootstock used was 'Gisela 6', that is not too vigorous and tree canopies are not too dense, allowing a better light penetration.

The favourable microclimate conditions observed under netting (Fig. 1) promoted a better tree water status, as demonstrated by the less negative values of stem water potential (Ψ_{stem}) and the higher P_n , and stomatal conductance (g_s) values observed in this treatment (Fig. 2). The lower VPDs observed under netting (Fig. 1), might have favoured a higher stomatal conductance for a longer period of time (Jutamane and Onnom, 2016; Mira-García et al., 2020), increasing leaves gas exchanges (Figs. 2B and 2C). This increase in the leaves' gas exchanges under netting promoted less negative Ψ_{stem} values (Fig. 2), confirming the positive effect of nets on tree water status. In line with an improvement in the water status, trees grown under netting also showed an increase on seasonal fruit growth (Fig. 3). This could be related to the fact that these trees showed a high photosynthetic capacity, which could have increased fruit fresh weight (Fig. 3), in accordance with what was reported previously by Garrido et al. (2023).

4.2. Effect of the net on the daily patterns of fruit transpiration, xylem, and phloem flows, and their relationship with the vapor pressure deficit

The daily contribution of fruit transpiration (T) flow increased from early stage II to early stage III of fruit growth (Fig. 10B), in agreement

with what was reported by Knoche et al. (2001). These authors reported how sweet cherry cuticle permeance increases progressively during these phenological stages, thus leading to higher fruit transpiration rates. In this study, the net induced a reduction in fruit T flow (Figs. 4–6B). However, in both treatments, T was closely related (r up to -0.91) with VPD (Fig. 7). This fact pointed out VPD as the primary driving force for water vapor diffusion fruit to the atmosphere, in agreement with what was previously reported on this crop by Brüggewirth et al. (2016) and Morandi et al. (2007). Then, the fact that the daily contribution of fruit T flow was significantly reduced under netting during late stage II and early stage III of fruit growth (Fig. 10B) was due to the fact that VPD was lower in this treatment (Fig. 1). In our trial, on both treatments, T flow became more negative as the VPD increased (Fig. 7), due to an absence of strong fruit stomatal regulation as a consequence of the low fruit stomatal density or the loss of stomata functionality during fruit development (Peschel et al., 2003).

At stage II of fruit growth, fruit water inputs occurred mainly via X (Figs. 4C and 5C), making this a key feature for recovering the water lost by T and allowing fruit cell division and expansion (Fig. 10B). However, the X contribution slightly decreased at the early stage III of fruit growth (Fig. 10C), in agreement with what is reported in other papers on sweet cherry (Brüggewirth et al., 2016), apricot (Giovannini et al., 2022) and grape berry (Bondada et al., 2005). This decrease in fruit X flux could be explained by a reduction in its hydraulic conductivity, possibly due to: (i) a physical disruption of the xylem from a growth strain and/or (ii) a blockage of the xylem in the pedicel (Winkler and Knoche, 2021).

As a consequence of the higher VPD (Fig. 1) fruit X flow was higher in control fruit (Figs. 4C, 5C, 6C, and 10C), even if the slope of the

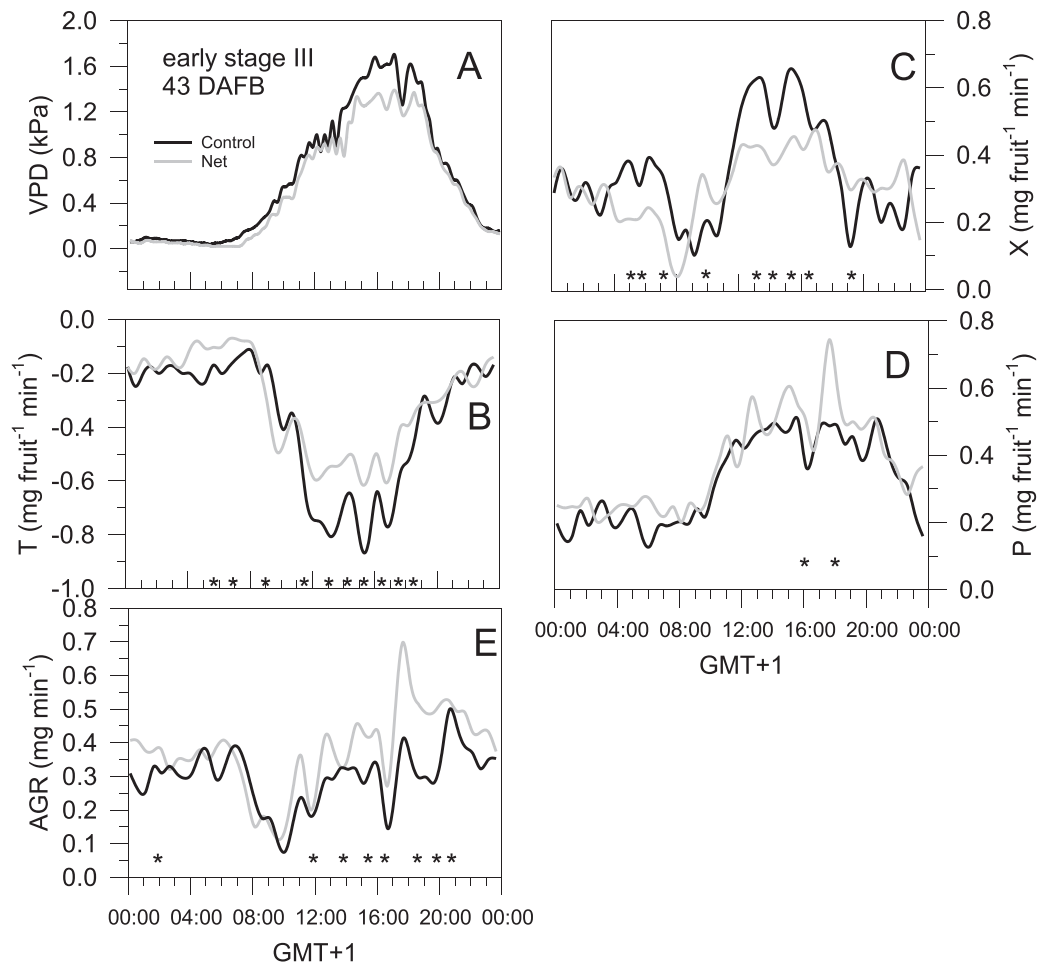


Fig. 6. Daily courses of vapor pressure deficit (VPD) (A), fruit transpiration (T) (B), xylem (X) (C), phloem (P) (D) in/outflows, and absolute growth rate (AGR) (E), at early stage III, in control and netted treatment. Data represent the 30-minute average values per treatment ($n = 4$). * indicates statistically significant differences between treatments according to the Mann-Whitney U test at $p < 0.05$. DAFB means days after full bloom.

relationship between VPD and X was similar between the two treatments (Fig. 8). But, during late stage II of fruit growth, the intercept of the relationship between VPD and fruit X flow was the highest in the control treatment (Fig. 8B). This indicates that for any given VPD fruit X flow was the highest in the control fruits. This could be due to the higher concentration of soluble solids in these fruits (data not shown), which could increase the fruit sink strength for xylem inflows.

In this study, fruit X flow as T flow were influenced by the VPD, (Figs. 7, and 8). At the same time, in our trial, VPD and fruit X flow were linearly related, except during early stage III of fruit growth for the control treatment (Fig. 8C). This could be a consequence of a loss of xylem functionality during this stage of fruit growth, in agreement with what was reported in apricot (Giovannini et al., 2022), in sweet cherry (Brüggenwirth et al., 2016) and in European plum (Winkler and Knoche, 2021).

Contrary to the fruit X flux, the P one increased continuously from early stage II to III of fruit growth (Fig. 10C), enhancing fruit sink activity. From both treatments, at early stage III, fruit P flow was the highest under netting, which could be due to on this treatment, the photosynthetic capacity of the trees was the highest (Fig. 2B), which led to a high carbon assimilation and photoassimilates production, and an increase in the transport of these products via phloem tissue. Confirming this idea, during early stage III, some significant differences were observed on the intercepts of the linear regressions, in such a way that, when VPD was equal 0, the fruit P flow was higher in the net than in the control treatment (Fig. 9C). This means that without the influence of environmental conditions, the transport of photoassimilates via P tissue

is related to tree photosynthetic capacity.

Among the three fruit flows, P was the least influenced by the meteorological conditions. In fact, it was only correlated with VPD at early stage III of fruit growth (Fig. 9C). This lack of effect of environmental conditions on the fruit P flow at stage II of fruit growth might be due to a predominance of apoplastic phloem unloading (Gao et al., 2003), since this mechanism is not dependent on the fruit-stem water potential gradients, which are affected by the environmental conditions. Whereas, at early stage III of fruit growth, apoplastic phloem unloading might be aided by some symplastic driving force, more dependent on the environmental conditions as was reported in plum (Grappadelli et al., 2019), which could justify the improvement in the relationship between VPD and fruit P flow.

4.3. Effect of the net on the daily patterns of fruit absolute growth rate

During the experiment, fruit absolute growth rate (AGR) increased progressively from early stage II to III of fruit growth (Fig. 10A), in agreement with fruit P flow, as during stage III, 50 %-80 % of the fruit growth occurs (Ayala and Lang, 2018). Also, at early stage III, fruit AGR was higher under nets (Fig. 10A), in agreement with the seasonal fruit growth (Fig. 3). This was mainly due to the higher fruit P flows recorded at this stage of fruit growth for this treatment, as a possible consequence of an increase in the photoassimilated availability linked to a high photosynthetic capacity of the plants cultivated under nets (Fig. 2B).

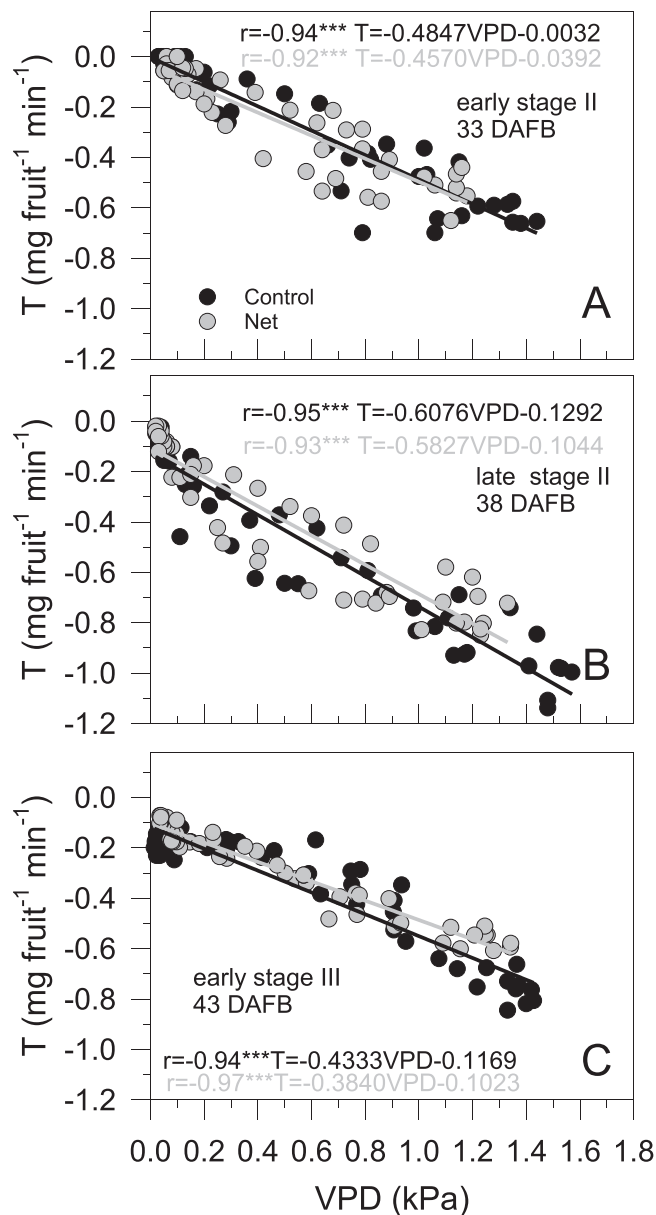


Fig. 7. Relationships between 30-minute data of vapor pressure deficit (VPD) and sweet cherry fruit transpiration (T) flow at early (A) and late stage II (B), and at early stage III (C) of fruit growth. Each point is the mean of 4 replicates. The level of significance of the relationships is shown as *** ($P < 0.001$). The linear equations of the VPD vs T were shown on the graphs. DAFB means days after full bloom.

5. Conclusions

In conclusion, the nets reduced air vapor pressure deficit within the canopy, having a positive effect on tree water status. Moreover, the nets affected the daily patterns and the total daily fruit transpiration, xylem, and phloem flows. Specifically, under netting, the contribution of these fruit flows was reduced, except in the case of the P, at the early stage III of fruit growth. At the same time, trees grown under nets showed a higher photosynthetic capacity, with positive effects on fruit growth. Moreover, in this study, the net slightly affected the relationship between the air VPD and fruit T, X, and P flows, which depended on the fruit growth stage.

Nowadays, nets are commonly used in sweet cherry orchards as they demonstrate real benefits against fruit disorders such as cracking and pests such as *Drosophila suzukii*. However, the effect of nets at the tree

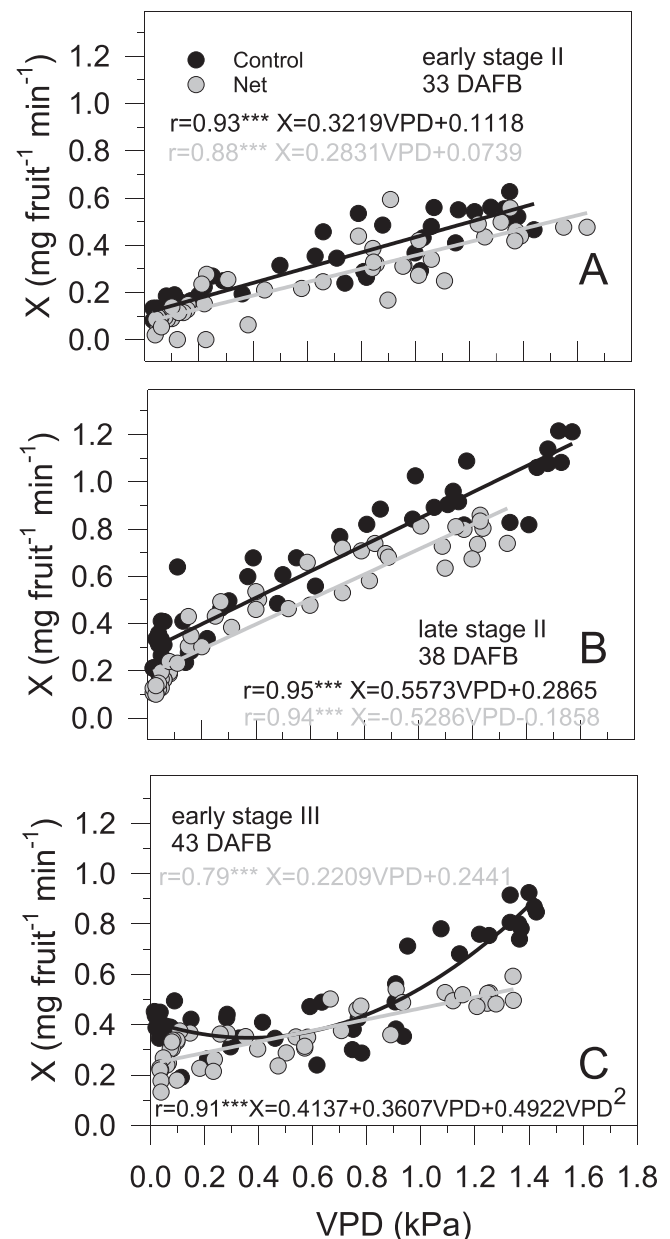


Fig. 8. Relationship between 30-minute data of vapor pressure deficit (VPD) and sweet cherry fruit xylem flux (X) at the early (A) and late stage II (B), and at the early stage III (C) of fruit growth. The regressions were adjusted for the two treatments studied: control and net. Each point is the mean of 4 replicates. The level of significance of the relationships is shown as *** ($P < 0.001$). The equations of the VPD vs X were shown on the graphs. DAFB means days after full bloom.

and fruit levels can vary depending on their material, shading level, crop variety, but also on the vigour of the rootstock used. In this sense, more comprehensive studies are needed to optimize the type of net based on the different features of sweet cherry orchards.

CRediT authorship contribution statement

Andrea Giovannini: Methodology, Data curation. **Melissa Venturi:** Methodology. **Rafael Dreux Miranda Fernandes:** Methodology. **Andrei Pasquali:** Methodology. **Mira García Ana Belén:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Brunella Morandi:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition,

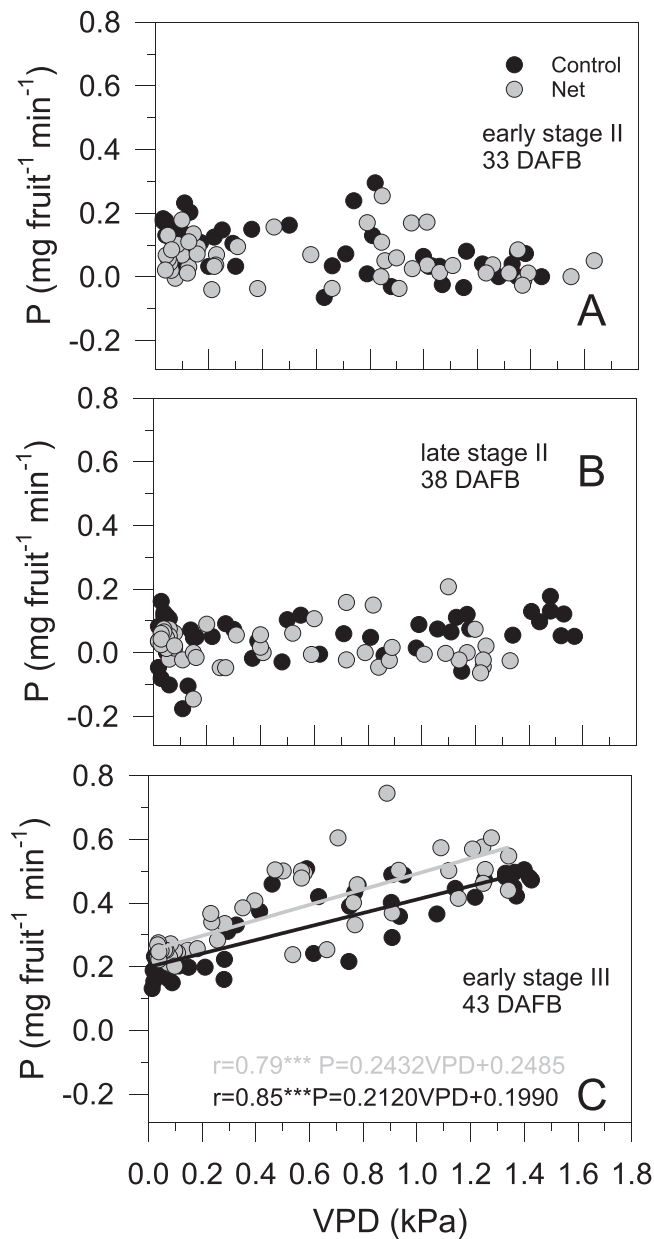


Fig. 9. Relationship between 30-minute data of vapor pressure deficit (VPD) and sweet cherry fruit phloem flux (P) at the early (A) and late stage II (B), and at the early stage III (C) of fruit growth. The regressions were adjusted for the two treatments studied: control and net. Each point is the mean of 4 replicates. The level of significance of the relationships is shown as *** ($P < 0.001$). The equations of the VPD vs P were shown on the graph. DAFB means days after full bloom.

Conceptualization.

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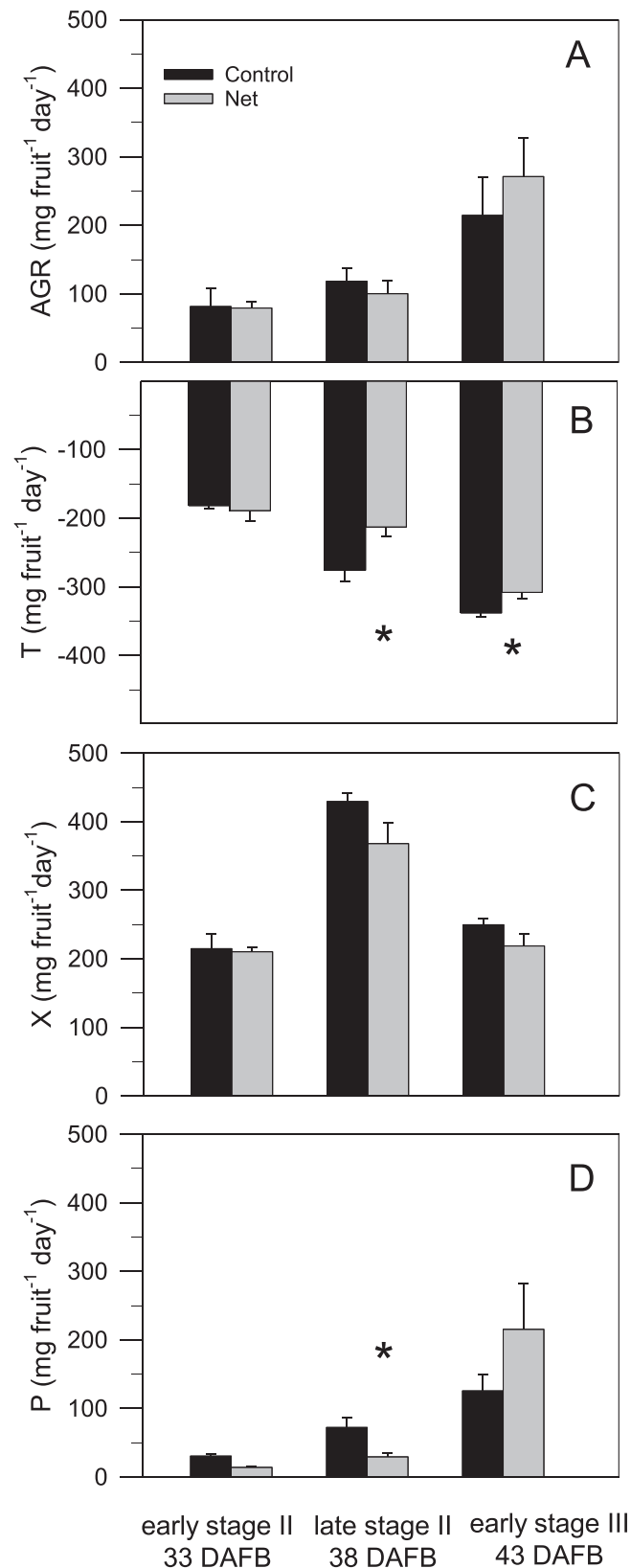


Fig. 10. Daily mean \pm standard error of fruit absolute growth rate (AGR) (A) and its transpiration (T) (B), xylem (X) (C), and phloem (P) (D) flows at the early and late stages II, and at the early stage III of fruit growth. Each bar is the average of 4 replicates. * indicates statistically significant differences between treatments according to the Mann-Whitney U test at $p < 0.05$. DAFB: days after full bloom.

Declaration of Competing Interest

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

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