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ADAPTING FRUIT PRODUCTION TO CLIMATE CHANGE: HOW TO INCREASE INTRINSIC FRUIT QUALIY AND SAVE WATER THROUGH DEFICIT IRRIGATION SCHEDULING

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ABSTRACT

Fruit crops are an important resource for food security, since more than being nutrient they are also a source of natural antioxidant compounds, such as polyphenols and vitamins. However, fruit crops are also among the cultivations threatened by the harmful effects of climate change. As droughts are becoming more and more common, water scarcity is expected to become the most limiting factor for fruit tree growth and productivity. Longer and more severe drought periods are expected, which will lead to the expansion of arid regions, which is particularly worrisome for the Mediterranean countries. In this context, knowing how plants and fruit are able to adapt to these stressors through physiological reactions it will be of extreme importance. This study had the objective of investigate the physiological effects of deficit irrigation on apple (2020-2021), sour cherry (2020-2021-2022) and apricot (2021-2022) trees, with a special focus on fruit nutraceutical quality. On each trial, the main physiological parameters were monitored along the growing season: i) stem and leaf water potentials; ii) leaf gas exchanges; iii) fruit and shoot growth. At harvest, fruit quality was evaluated especially in terms of fruit size, flesh firmness and soluble solids content. The analysis on fruit sampled at harvest were: i) total phenolic content determination; ii) anthocyanidin concentration evaluation; and iii) untargeted metabolomic study.

On apple, trees subjected to a deficit irrigation (50% of Etc) showed a decrease in stem water potential values, leading to a decrease in final fruit size at harvest during the first year of trial. Thanks to the vascular inflows at earlier timings and to a reduction in transpiration fruit subjected to deficit irrigation were able to maintain growth rates similar to those of fruit from control plants, although fruit size at harvest was negatively affected. Moreover, the comparison of fruit vascular flows at 107 DAFB in two different years showed that xylem disfunction is related to the seasonal weather conditions, which was able to anticipate or delay the xylem dysfunctionalities typically occurring during the second part of the season. Fruit total phenolic content was not influenced by deficit irrigation, probably because the stress imposed was not sufficient to activate an up-regulation in the synthesis of specialized metabolites. On sour cherry, plants managed to resist to rainfed conditions without

showing particularly intense deficiency and without compromising their physiological functions. Moreover, fruit soluble sugar content was not affected by the irrigation treatment applied while total phenolic content was increased in non-irrigated trees. Water strees deeply modified fruit metabolomic landscape with an increase in lipids and carbohydrates as well as a drecrease in cinnamic and carboxylic acid and terpenoid metabolites. For these reasons, the lack of irrigation in the last weeks before haverst can be successfully applied on sour cherry, without penalizing plant performances and could also be used as a tool to modulate the fruit metabolome. On apricot, trees were able to afford a long dry period without showing significant decreases in plant physiological performance as well as fruit growth over two years of study. This might be due to a possible “stress memory” but for sure the use of vigorous rootstocks characterized by deep root systems enhanced tree resilience to water stress. In addition, deficit irrigation seems to improve fruit quality in terms of sugar content but, unfortunately, the results shown in this study do not provide strong evidence supporting this relation. In conclusion, irrigation scheduling in apricot, apple and sour cherry is surely overestimated by the decision support system available in Emilia-Romagna region. Deficit irrigation protocols should be developed to optimize water use, fruit production and quality, especially in terms of nutraceutical components. The water stress imposed on different fruit crops, each during two years of study showed as a general conclusion that the decrease in the irrigation water did not show a straightforward decrease in plant physiological performance. This can be due to the miscalculation of the real water needs of the considered fruit crops. For this reason, there is the need to improve this important tool for an appropriate water irrigation management. Furthermore, there is also the need to study the behavior of fruit crops under more severe deficit irrigations. In fact, it is likely that the application of lower water amounts will enhance the synthesis of specialized metabolites, with positive repercussion on human health. These hypotheses must be verified.

LIST OF ABBREVIATIONS

ABA: abscissic acid

DAFB: days after full bloom

DI: deficit irrigation

DHFI: deficit high-frequency irrigation

Etc: crop evapotranspiration rate

PRD: partial root-zone drying

RDI: regulated deficit irrigation

SDI: sustained deficit irrigationSM: specialized metabolites

SSC: soluble solids content

TPC: total phenolic content

TSS: total soluble solids

VPD: vapour pressure deficit

WUE: water use efficiency

CHAPTER I.

INTRODUCTION AND AIM OF THE STUDY

Perennial fruit crops are among the species susceptible to the negative effects of climate change. Droughts are occurring more frequently and among abiotic stress factors, water scarcity is anticipated to have the worst effect on crop growth and productivity in the future (Shao et al., 2008). This trend is especially concerning for the Mediterranean regions, where there will be more frequent periods of severe drought, which will cause arid areas to grow (Giorgi and Lionello, 2008). A common strategy to overcome the negative effects of water scarcity is irrigation which is used normally in countries where the lack of water is not a serious problem yet. Irrigated agriculture uses the majority of available water, accounting for between 70 and 80 percent of the total water available in arid and semi-arid regions (Fereres and Soriano, 2007). However, worldwide water supplies are becoming scarce (Postel 1998). In areas with limited water resources, irrigation will need to be reduced. Better irrigation scheduling for fruit crops is required due to the global decline in freshwater availability for horticulture use (Rouphael et al. 2008). However, water conservation in irrigation should not come at the expense of crop yield or product quality. This is especially true for fruit crops whose quality will win the preference of consumers. These climate changing conditions have a tendency to reduce the potential for fruit growth, making it more difficult for growers to meet the quality standards demanded by the market in terms of fruit size and productivity.

DEFICIT IRRIGATION: DEFINITIONS

It is necessary to define the full crop evapotranspiration (ETc) requirements in order to define the level of deficit irrigation to impose. Research on crop water requirements has produced several valid methods for its calculation since Penman (1948) developed the combination approach to calculate plant evapo-transpiration rate. The Penman-Monteith equation is currently the accepted method for

determining the ETc of the major herbaceous crops with sufficient precision for management purposes (Monteith and Unsworth, 1990; Allen et al., 1998). However, there is more uncertainty when applying the same methodology to determine the ETc requirements of vines and tree crops which often lead to an overestimation of the water used (Fereres and Goldhamer, 1990; Dragoni et al., 2004; Testi et al., 2006). However, it is clear that in a climate changing scenario with decreased water availability, is no longer affordable to supply fruit crop in accordance to the Etc. For this reason, researchers have started studying the effects of the application of reduced irrigation volumes on plant physiology. According to the literature, different types of water deficit can be imposed to fruit tree crops. Here below we regroup them in 3 major categories:

- **Deficit irrigation (DI)**. It is a method of controlling soil water supply to impose predetermined plant or soil water deficit periods that may have some economic benefit (Mills et al., 1997). It entails providing the plant with less water than the crop evapotranspiration (ETc) demand at specific times throughout the growing season.
- **Regulated deficit irrigatio**n (RDI). This term is typically used when the deficit irrigation is applied early in the season, before the beginning of rapid growth phase. Late-season RDI is when DI is applied toward the end of the growing season but prior to harvest; its duration depends on the objective, species, and environmental factors. It was first used as a management strategy to control vigour in high-density plantings of peach (Chalmers et al., 1981) and pear (Mitchell et al. 1984). The protocol then foresees to provide water at the beginning of the cell expansion stage, but at a lower rate than ETc.
- **Sustained deficit irrigation (SDI).** It consists in the application of reduced irrigation volumes at regular intervals throughout the entire growing season. As a matter of fact, this practice was initially tested as deficit high-frequency irrigation (DHFI). It is common practice to apply the SDI to young plantations to evaluate the impact of potential moisture stress on the vegetative growth and fruiting of young trees (De Oliveira et al., 2017; Treder et al., 2004; Ucar et al., 2016). This method can also be used to readjust the traditional crop Kc

models (Allen et al., 2005) on the basis of particular orchard conditions (Chauhan et al., 2005; Naor et al., 2008; O'Connell & Goodwin, 2007).

- **Partial root drying (PRD).** At each irrigation episode, only a portion of the root zone is watered; the remaining portion is allowed to dry to a predetermined level of soil moisture (Dry and Loveys 1998). The previously unirrigated area of the root zone will receive the subsequent irrigation. This stimulates roots in drying soil to produce chemical messages (like abscisic acid) that are transported to the shoots and cause stomata to partially close. As a consequence, transpiration will decrease along with some degree of photosynthesis. Plant water potential will presumably be higher than in classical DI, since plant water potential will be expected to equilibrate with the wetter part of the rootzone, the irrigated part (Hsiao, 1990).

THE EFFECTS OF DEFICIT IRRIGATION ON PLANT PHYSIOLOGY

In order to deal with water shortages, plants have evolved physiological reactions as well as ecological strategies, such as stress tolerance or stress avoidance. Thanks to these reactions plants are able to survive and even continue to grow. The type of water shortage will determine the plant reactions, with physiological responses to short-term changes (Hsiao et al., 1976), acclimation to a specific level of water availability (Schulze, 1986), and adaptation to drought (Levitt, 1980; Alscher and Cumming, 1990). Knowing the mechanisms that help plants resist drought allow to effectively plan deficit irrigation strategies to conserve water while minimizing the negative effects on yield or crop revenue (Domingo et al., 1996).

Water relations

Water is a crucial component for cell turgor pressure maintenance and it is a necessary medium for biochemical processes. Additionally, a water shortage results in dehydration, which has a detrimental impact on a plant's metabolism and survival (Dwivedi and Dwivedi, 2012). When more water is lost by transpiration than what is absorbed from the soil, plants become stressed (Kramer, 1989).

Orchard irrigation can be optimized using the stem water potential as physiological indicator, since usually this is the first parameter to respond when plants are subjected to short- and long-term drought periods (Shackel, 2007). The onset of drought conditions rapidly modifies tree water relations by decreasing either leaf or/and stem water potentials, depending on a wide range of species-specific hydraulical features such as vessel size, density, etc. (Dichio et al., 2013; Marsal et al., 2008; Lo Gullo et al., 2003; Fernandez et al., 2001; Naor et al.,1995; McCutchan and Shackel, 1992).

Tree water relations normally change throughout the day in response to environmental changes: stem, leaf and fruit water potentials always show a decline at noon, followed by a recovery in the late afternoon and evening (McFadyen et al.,1996; Morandi et al., 2010a). According to Liu et al. (2006) and Pérez-Pastor et al. (2014), plants may be encouraged to significantly reduce leaf water potential during a brief period of mild water deficit under RDI. Along with the chemical signals, decreased leaf water potential serves as a hydraulic signal that triggers reduced leaf area expansion and partial stomatal closure (Shahnazari et al.2007). These changes in leaf water potential can therefore indirectly affect the transport of water and assimilates to developing fruit because the water potential gradients among these organs are the drivers for phloem and xylem flows in the tree vascular path (Münch, 1930).

When possible, in order to avoid excessive stresses that would result in lower yield and fruit quality, stem water potential thresholds have been set from previous studies; for example, a threshold of -2 MPa has been proposed as a reference value for drought stress in citrus (Gasque et al., 2016) while for apple it has been fixed at -1 MPa (Naor et al., 1995; Naor, 2012; Lopez et al., 2018; Boini et al., 2019).

Leaf gas exchanges

Stomata regulate plant ability to absorb CO_2 and to release water vapour. Soil water is effectively mobilised via stomatal transpiration into the air due to the gradient of water potential from the soil to the plant and on to the atmosphere. Constraints on this water flux are imposed by factors such as the availability of water and the plants' ability to conduct water.

For this reason, a key process in determining WUE in plants is the prompt regulation of stomatal closure. Although there is a non-linear relationship between stomatal conductance and photosynthetic rate, the linear relationship between stomatal conductance and transpiration under constant vapor pressure deficit (VPD) suggests that a higher stomatal closure may result in more efficient water use (Chaves et al., 2002). Recent experimental evidence calls into question whether root-derived ABA is the sole or even primary cause of stomatal closure in drying soil (Buckley, 2019; Yoshida et al., 2019a,2019b; Zhang et al., 2022). ABA has long been known to be synthesised in dehydrating tissues (LOVEYS, 1977) and to induce stomatal closure (Kriedemann et al., 1972). For many years, it was assumed that ABA produced in drying roots and transported to leaves via the xylem was primarily responsible for stomatal closure during soil drought. However, hormonal signals (e.g. cytokinins, ABA) together with circadian rhythms and leaf water status are just a few of the factors that can have an effect on stomatal closure. All these factors are integrated to deliver a specific stomatal conductance according to the environmental situation experimented by the plant at any given moment (Webb and Hetherington, 1997; Bacon, 2004). Changes in stomatal conductance in response to temperature are strictly connected to internal plant processes like carbohydrate metabolism and enzymatic activity as well as external ones like air VPD. Stomatal conductance usually peaks in the 20°C - 40°C temperature range and drops off dramatically at temperatures as low as 5°C or as high as 45°C (Stalfet, 1962; Jarvis, 1976). Stomatal closure represents the most immediate physiological response to drought stress in woody plants (Damour et al., 2010) and contributes to the decrease of leaf transpiration. Each fruit tree crop can react in different ways and influence leaf gas exchanges as a consequence of water stress. As an example, we can cite two opposite response mechanisms that take place in fruit crops. On one side, kiwifruit exhibits stomatal closure at the onset of water stress in order to minimize water losses and maintain its water potential in a "safe" range (i.e., high enough) to prevent the occurrence of cavitation events in the xylem, that would impair its hydraulic function (Morandi et al., 2010). One the other side, olive is capable to maintain leaf transpiration under drought conditions thanks to their anatomical features, such as high wax content to increase cuticle diffusion resistance, high trichome presence that

provides a better control of transpiration as well as thin xylem vessels that reduce the risk of embolism (Carr, 2013). The control of stomatal behaviour under drought conditions have also been seen in grapevines (Pou et al., 2008) and kiwifruit (Buckley, 2005; Galmés et al., 2007; Torres-Ruiz et al., 2013).

However, although the reduction in transpiration aids the plant in maintaining water balance, this implies a decrease in net CO2 uptake as well. In addition to reducing the amount of carbohydrates available to the fruits, a decline in net photosynthesis (Kramer, 1989) also fosters the development of photo-oxidative stress, or the photosynthetically associated production of reactive oxygen species (ROS), such as O2-, •OH, and possibly H2O2 (Grassmann et al., 2002).

Shoot seasonal growth

Deficit irrigation was initially developed to control vegetative growth. In fact, it was observed that a reduced amount of water supplied led to a decreased vegetative production, with consistent savings during pruning operations (Chalmers et al., 1981; Mitchell et al., 1984). From a general point of view, the strength of the shoot growth rate is directly related to the strength of the roots growth rate (Wareing, 1950). For this reason, dwarfing rootstocks are generally employed to reduce plant size and vegetative growth (Luckwill, 1969). The interaction between rootstock vigour and deficit irrigation was studied recently by Venturi et al. (2021). In this study, it emerged that shoot growth was not affected by deficit irrigation, while plants grafted on vigorous rootstock showed a decrease in shoot growth when receiving 60% of ETc. This confirms that deficit irrigation affects vegetative growth, however also the scion-rootstock interaction can strongly influence shoot vegetative growth.

Sap flow

The study of the water transfer from soil to plants and to the atmosphere is known as the soil-plant-atmosphere continuum (SPAC), which is considered as the most important theoretical framework for comprehending plant water movement (Deng et al., 2017). Plant water potential, soil moisture content, plant uptake, evapotranspiration and dynamics of xylem transport are the SPAC most important elements (Vicente et al., 2003;Fayer and Gee, 2004;Vidana Gamage et al., 2021). In

plants, the stem sap flow serves as a measure of transpiration and water use. Heat balance and thermal dissipation are the foundations of the most widely used techniques for measuring sap flow. However, these are invasive procedures that are prone to a variety of mistakes, such as probe misalignment (Ren et al., 2017). Proposed methods measure either flow rate (g h^{-1}) or flux density ($cm^3cm^{-2}h^{-1}$) to calculate the amount of sap flowing through a surface per unit time (Vandegehuchte and Steppe, 2012). Thermodynamic, electrodynamic and magneto dynamic concepts are the foundation of various methods for measuring sap flow. Thermodynamic-based techniques are among the most popular ones that are available (Ermáket et al., 2004). There are three main categories of thermodynamic techniques for measuring sap flow:

- Heat balance method involves wrapping the stem in foam insulation and a thin, flexible heater (Sakuratani, 1981). This method estimates heat loss due to the mass flow of water in the stem and takes both conduction and convection losses into account (Sakuratani and Abe, 1985).
- Thermal dissipation method uses two needles 10 cm apart that are mounted with thermocouples to measure convective heat transfer (heat carried by the sap stream).
- Heat pulse velocity method, detects the passage of the heat pulse through the xylem.

Since sap flow is considered among the main plant physiological indicators, its monitoring during the day and during the growing season is usually employed to understand tree physiological status under different stressful conditions. Studies are available on apple (Nadezhdina1999), grapes (Escalona et al.2002), lemon (Ortuo et al.2006), and plum (Intrigliolo and Castel2006). In addition to using sap flow records to analyse the dynamics of plant transpiration, different approaches to planning irrigation have also been put forward. To manage irrigation in fruit tree orchards, Nadezhdina and Cermak (1997) developed what they called the Sap Flow Index (see Nadezhdina 1999 for details). It has been proposed that a subtle change in the sap velocity profile's shape caused by water stress would be a suitable indicator for starting irrigation. Sap flow rate was not affected when the irrigation is applied at different timings during the day, in kiwifruit (Torres-Ruiz et al., 2016). However, it is

important to note that sap flow is closely related to environmental condition and it shows great variability in response to the VPD experienced (Saitta et al., 2021). In fact, when air temperature and VPD increase the first plant response is stomatal closure with following sap flow decrease (Consoli et al., 2014). This tree response is even more exacerbated when deficit irrigation is applied, contributing to the decrease of sap flow rates (Saitta et al., 2021; Espadafor et al., 2017; López-López et al., 2018; Roccuzzo et al., 2014a, b).

Fruit growth, vascular flows and transpiration rate

Fruit growth can be described as the alternate shrinkage and swelling of the fruit, resulting in an irreversible increase in size and weight (Boini et al., 2019; Lang, 1990b; Morandi, Rieger, et al., 2007b; Fishman and Génard, 1998).

Fruit transpiration and fruit respiration are the primary outgoing fluxes, whereas water and assimilates are transported to the fruit via xylem and phloem streams, respectively. Fruit trees have a variety of defences against water stress. For instance, by closing their stomata in the middle of the day, leaves can draw water from fruit (Westwood, 1993). Some fruit species, including citrus (Mantell et al., 1980) and apple (Lang, 1990), have demonstrated that a reverse water flow from fruit to leaves is also possible via xylem backflow. The net contribution of phloem import (which is always positive) xylem flow (which may or may not be positive) and transpiration through the cuticle (which is always negative), can be seen as the cause of the variation in fruit diameter over a finite period of time. Turgor pressure and osmotic concentration gradients along the vascular pathway from source to sink organs drive phloem and xylem flows into the fruit (Munch, 1930; Minchin and Thorpe, 1987; Patrick, 1990, 1997; Minchin et al., 1996). McFady et al. (1996) reported diurnal patterns of leaf and fruit water potentials in peaches and linked changes in fruit growth rate over 24 hours to shifting water potential gradients between fruit and leaves. According to Higgs and Jones (1984) and Jones and Higgs (1985), there is a strong correlation between shrinkage (which happens during the day) and environmental factors in apples. Fruit shrinkage generally happens as a result of fruit transpiration and appears to play a significant role in lowering fruit water potential, which in turn creates the conditions for subsequently loading the fruit with dry

matter (Jones and Higgs, 1982; Berger and Selles,1993; McFadyen et al., 1996). It has been observed in pear how decreases in water supply first decrease fruit xylem inflow and then fruit phloem inflow, resulting in a higher fruit dry matter accumulation (Morandi et al., 2014)

Fruit transpiration is a physical process that relies on both external factors and epidermal characteristics. Vapour pressure deficit (VPD) drives it and its magnitude is inversely related to the fruit surface area and skin permeability coefficient (Lescourret et al., 2001; Wuet al., 2003). In many crops, when fruit are close to harvest, specific transpiration is significantly reduced. It has been revealed that the cuticle thickens in apples (Jones and Higgs, 1982; Lang, 1990) while peaches continue to lose water at higher specific rates (Lescourret et al., 2001; Li et al., 2002; Wu et al., 2003). As a matter of fact, young fruits lose more water than mature ones through transpiration, releasing a greater quantity of xylem sap, and for this reason they are more likely to maintain a symplasmic phloem unloading (Rossi et al., 2022). Fruit surface conductance is strictly related to fruit transpiration. Fruit with a higher surface conductance can draw more water (and probably mineral elements) from the xylem, thereby providing the physical and biological conditions necessary to support passive phloem unloading. As a result of reduced water exchanges caused by transpiration, apoplasmic phloem flow is more common in fruits with low surface conductance (Rossi et al. 2022).

THE EFFECTS OF DEFICIT IRRIGATION ON FRUIT QUALITY

Fruit quality is determined by a number of factors, the relative weight of which varies depending on the producer, consumer and distributor (Shewfelt, 1999). Commercial quality is determined by fruit size and exterior appeal (colour, form, size, etc.), whereas adequate flesh firmness ensures an extended shelf-life. Fruits are primarily judged on their sensory qualities, such as their total soluble solids or sugar content, sweetness, titratable acidity, freshness, and colour (Nora et al. 2012). Additionally, the nutritional value of fresh fruits, which is typically correlated with their antioxidant content (mainly polyphenols and vitamin C), is a characteristic of increasing importance for consumers.

Although one of the main concerns when applying deficit irrigation is related to the effects on yield, also fruit quality can be impacted (Torrecillas et al., 2000; Pérez-Pastor et al., 2007a).

Fruit size

Water potential gradients between the stem and the fruit are responsible for driving vascular flows that sustain fruit growth (Fishman and Gènard, 1998). This means that the application of deficit irrigation, which is known to influence water potential gradients inside the plant, also fruit growth can be affected. For this reason, the effects of deficit irrigation on fruit size has been deeply studied. The majority of studies pointed out how water stress negatively affects fruit size, especially when applied during the cellular expansion phase on apricot (Torrecillas et al., 2000), plum (Naor et al., 2004), pear (Morandi et al., 2014; Venturi et al., 2021), pomegranate (Peña et al., 2013) and apple (Gariglio et al., 2007; Ebel et al.,1993). Crop load reduction can be a strategy to smooth this detrimental effect (Naor et al., 1999; Wünsche et al., 2000). Fruit size is a characteristic that depends also on the plant vigour. It was found that plants grafted on dwarfing rootstocks are more sensitive to deficit irrigation, leading to a reduced fruit size at harvest (Venturi et al., 2021). Light management has been recently proven to be a key point to obtain fruit of optimal size under deficit irrigation conditions (Boini et al., 2022). Although shading is not always considered to increase fruit quality, light diffusion with white nets can improve leaves efficiency at whole canopy level, mitigating the negative effects usually related with lower irrigation volumes (Boini et al., 2022).

Fruit colour

As for fruit size, the effect of deficit irrigation on fruit colour development are not always in accordance. Several studies showed that a decreased irrigation led to an enhanced coloration of the fruit (Torrecillas et al., 2000; Pérez-Pastor et al., 2009 ;Mills et al. 1994; Kilili 1996a, b;). This might have been caused by the advanced accumulation of sugars found in these fruits, as sucrose is crucial for the production

of anthocyanins, in red-skin fruit. However, the higher colour intensity can also be related to and advancement in fruit maturity (Torrecillas et al., 2000).

When weather conditions are not favourable for fruit colour development (e.g. high temperatures during the night) deficit irrigation alone is not sufficient for fruit colour development (Mpe-lasokaetal.2001b; O'Connell and Goodwin (2007a Talluto et al. (2008)). When the environmental factors are favourable for colour development, reduced irrigation can lead to the enhancement of fruit colour. Another possible factor influencing fruit colour development is the amount of light that reaches the fruit, able to positively determines both red and yellow coloration in apple (Boini et al., 2022; Lancaster 1992). Under deficit irrigation, less vegetative growth might allow more light to enter the canopy and an appropriate net for light diffusion can even improve the fruit overall coloration.

Fruit flesh firmness

Fruit maturity has a significant impact on fruit firmness, since it decreases as the fruit ripens (Kingston 1991). Fruit size affects firmness (Ebel et et al. 1993); smaller fruit tends to be firmer than larger fruit because of a higher cellular density. Therefore, when deficit irrigation applications change fruit size, also fruit firmness can be affected. Information on the impact of soil moisture on fruit firmness was ambiguous. Some studies found flesh firmness to be reduced in fruit from plants subjected to deficit irrigation (Drake et al. 1981; Mills et al., 1994;) advancing the hypothesis that fruit grown in dryer conditions ripen quickly. On the other hand, other researchers demonstrated that fruit from non-irrigated plots were firmer than those from irrigated plots (Guelfat-Reich et al. 1974; Assaf et al. 1975; Guelfat-Reich and Ben-Arie 1979; Cabral et al. 1995; Mpelasoka et al. (2001; Pérez-Pastor et al., 2007b ;Leib et al. (2006); Kilili et al. 1996b; Mpelasoka et al. 2000). Studies conducted on apricot showed that the firmness remained higher in DI fruit also after a period of storage (Torrecillas et al., 2000; Leib et al. 2006). A rise in cell fragility and size inflation may be the cause of the reduced firmness of fruit from well-irrigated trees (Guelfat-Reich and Ben-Arie 1979). However, some experiments showed that deficit irrigation had no effect on fruit firmness (Naor at al 2004; O'Connell and Goodwin 2007a; Talluto et al. 2008). Considering all the above mentioned we can conclude that in the majority of

the cases fruit firmness increases in response to DI, despite potential differences according to the cultivar considered.

Soluble solid content

The primary building blocks of plants are sugars, which also give them energy for cellular growth and reproduction. The three sugars that have the greatest concentrations in ripe fruits—sucrose, fructose, and glucose—are directly related to fruit taste (Romero et al., 2021). The main sugar that is transported from photosynthetic tissues to sink organs, like fruits, is sucrose (Durand et al., 2018), even though several other carbohydrates (i.e. sorbitol) can be translocated depending on the species. Once in the fruit, sucrose can either hydrolyse into hexose (fructose and glucose) or act as a precursor to the synthesis of carbohydrates polymers like cellulose and starch (Durán-Soria et al., 2020). Moreover, the ripening process is driven by crosstalk between sugars, particularly sucrose, and phytohormones, making carbohydrates key molecules in fruit development and maturation (Durán-Soria et al., 2020). Furthermore, sugar concentration is also related to the synthesis of secondary metabolites, as it promotes the accumulation of the anthocyanins and carotenoids involved in fruit appearance (Giampieri et al., 2015; Pott et al., 2019). Numerous authors, as reviewed by Naor (2006), claim that fruit total soluble solids content increased significantly under DI. Later publications continue to validate this observation in various fruit crops including apricot, plum, grape, citrus, pear and apple (Pérez-Pastor et al., 2007b ; Pérez-Pastor et al., 2009 ; Cabral et al. 1995 ; Mpelasoka et al.2001c; Naor et al 2004; Venturi et al 2021; Leib et al. 2006 ; Lopez et al. 2011 ; Treeby et al. 2007 ; Velez et al. 2007 ; Du et al. 2008)

However, some studies showed a decrease or no influence induced by deficit irrigation, as presented for citrus, grape, apple, pear and apricot (Dry 2005 ; Molina-Ochoa et al., 2015; Torrecillas et al., 2000; O'Connell and Goodwin, 2007a; Behboudian and Lawes 1994; Castel and Buj 1989).

The literature shows convincingly that fruit from plants subjected to deficit irrigation have a higher level of total soluble solids than control fruit, despite the possibility of a cultivar or a scion/rootstock effect in this regard.

THE EFFECTS OF WATER STRESS ON FRUIT NUTRACEUTICAL COMPOUNDS

The integrity of food supply has long been both of great interest and concern in civilized cultures. Before nutrition became a distinct scientific field, philosophers first and doctors later, paid special attention to how the daily food affected both personal and societal health. The intelligent selection of natural food products was a substantial part in the practice of medicine. It is interesting to note that for over 2000 years there was not a sharp distinction between food and drug (Andlauer and Fürst, 2002). Hippocrates wrote that "...differences of diseases depend on nutriment", acknowledging the fundamental connection between food and health (Jones, 1923a, 1923b).

Dietary substances that provide both nutrition and health advantages when consumed can be defined as nutraceuticals (Maurya et al., 2020). This term was coined in 1989 by the Foundation for Innovation in Medicine (New York, US) to provide a name for this rapidly expanding area of biomedical research. A nutraceutical is defined as any substance either food or part of a food that provides medical or health benefits such as disease prevention and treatment (DeFelice, 1992). Nutraceuticals may include isolated nutrients, dietary supplements, as well as genetically engineered foods, herbal products and processed foods such as cereals, soups, and beverages (Andlauer and Fürst, 2002). Nutraceuticals are referred as substances produced by primary and secondary metabolisms. Primary metabolites are essential for plant cell growth and development and are conserved across the plant kingdom (proteins, carbohydrates, lipids, nucleic acids). Specialized (secondary) metabolites are bioactive organic molecules with a low molecular weight, which are essential for plant interaction with the environment (Pichersky and Lewinsohn, 2011; Arimura and Maffei, 2017; Maeda, 2019; Corso et al., 2021; Baud et al., 2023).

Nutraceuticals are classified into seven classes based on their biosynthesis and origin, usage, and production methods:

- Functional food. The term functional food was first used in Japan in the 1980s to describe processed foods that contain ingredients that aid specific body functions in addition to being nutritious (Kaur and Das, 2011).

- Dietary supplement: they contain one or more concentrated nutrients with the goal of supplementing an individual's daily diet when is characterized by nutritional deficiencies. Dietary supplements are not typical foods, medicines, or special dietary products and they are not meant for specific groups of people. The majority of these supplements are available in tablet or powder form (Egbuna and Dable Tupas, 2020).
- Medical food: they are food items tailored to fulfil the nutritional demands of people who have specific illnesses or critical health conditions (e.g. hidden hunger). Medical foods, unlike other types of nutraceuticals, are consumed under the supervision of a qualified medical practitioner.
- Dietary fiber: they are edible but nondigestible parts of food that pass through the human small intestine. Except for lignin fiber, which are non-carbohydrate in nature, dietary fiber are carbohydrate in origin and made of 10 or more monosaccharide units.
- Farmaceuticals: they are plants or animals products that have been genetically engineered using biotechnology to provide additional health benefits in addition to nutrition. The term also refers to a beneficial substance derived from genetically modified agricultural crops or animals. These genetically engineered crops or animals have extra genes that make them rich in specific nutrients, enzymes, metabolites, or phytochemicals that can be used as medicine.
- Probiotics: they are groups of microorganisms that, when consumed in sufficient quantities, provide numerous health advantages to the host. Elie Metchnikoff (Nobel laureate, Pasteur Institute, Paris) demonstrated the beneficial impact of probiotics for humans when he successfully turned toxic microflora of the large intestine into a host-friendly colony of Bacillus bulgaricus, resulting in enhanced host health.
- Prebiotics: they are insoluble dietary fiber that pass through the small intestine undigested but are fermented by gut microorganisms once they reach the large intestine. They provide nourishment for probiotic bacteria, particularly Bifidobacterium. For this reason, they act in an indirect way without supplying sustenance to the host.

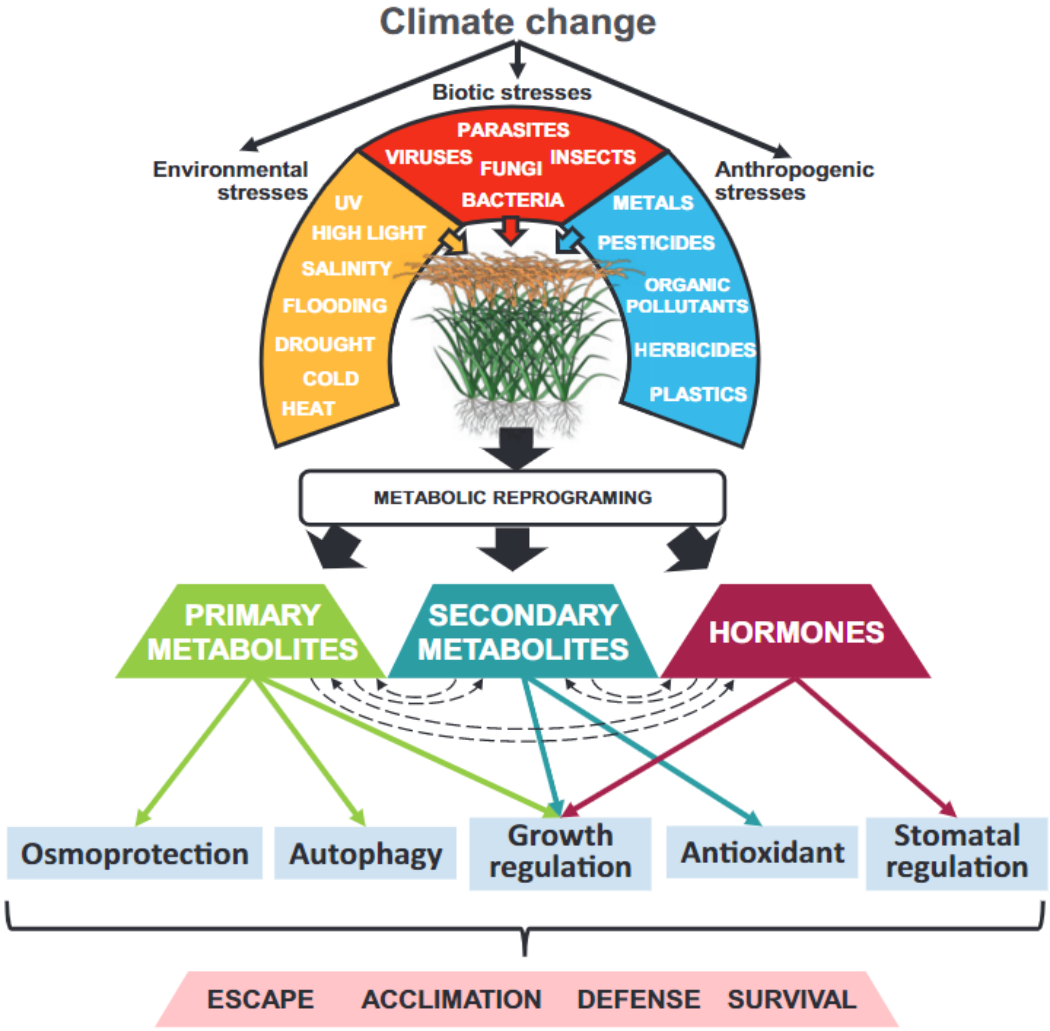

Figure 1. Multiple stress factors, including environmental, biotic, and/or anthropogenic stresses, may simultaneously impact plants in the field. Plants respond by reprograming their metabolic networks, accumulating specific primary and secondary metabolites as well as different hormones. The integration of different metabolic and hormonal pathways (dashed arrows) allows plants to adjust their growth and adopt a survival, escape, acclimation, and/or defense strategy. From Zandalinas et al., 2022.

The classification of the above-mentioned compounds is still lacking a well-defined and widely accepted classification. The definitions given in the literature are often differently expressed as well as the classifications of the bioactive compounds can be subjected to different rankings of importance.

Fruit, as natural product, are a source of nutraceuticals, thanks to their richness in polyphenols, phytoestrogens and many other antioxidants (Slavin and Lloyd, 2012). Fruit and fruit extract consumption reduce the risk of chronic diseases, which has been partially linked to the presence of specialized (secondary) metabolites (phytochemicals) with a wide range of biological activities. Research on apple (Hyson, 2011), quince (Al-Zughbi and Krayem, 2022), passion fruit (Fonseca et al., 2022) and date fruit established a link between their consumption and following health benefits. When compared to pharmaceutical drugs, specialized metabolites have low potency as bioactive compounds, but since they are ingested regularly and in significant amounts as part of the diet, they may have a noticeable long-term physiological effect (Espín et al., 2007; Bondonno et al., 2019; Yang et al., 2022). However, they are known to be substantially safer and with fewer side effects than many medicines.

Plants are able to synthesize over 200 000 specialized metabolites (Fernie et al., 2004; Pichersky and Lewinsohn, 2011; Corso et al., 2020). However, these numbers could be much higher since only a small portion of all the chemical molecules synthesised by plants have been annotated and characterized so far. These natural products are volatile, semi-volatile, and non-volatile chemicals formed from aminoacids, isoprenoids, nucleotides, and sugars, which are produced via primary metabolic pathways (Wink, 2010). They have long been considered as waste products of the metabolism, and the name "secondary metabolites" stems from the initial observations that, in contrast to primary metabolites, they were considered as compounds with unclear or no function (Hartmann, 2007) (Figure 1). We now refer to these compounds as specialized metabolites (SMs). Unlike the evolutionary limited primary metabolites, SMs rapidly evolve to allow plants to better react to environmental stimuli (Wagner et al., 2004; Weng et al., 2012). These compounds are capable to impact colour, taste, and flavour, but they can also be poisonous to other organisms and discourage herbivores and pathogens, protect from UV light, cold temperatures, drought (Wagner et al., 2004; Beran et al., 2019), or be attractive to pollinators and disseminate seeds. Hence, they are essential to mediate the plant interaction with its environment(s). Based on their biosynthetic and metabolic pathways, SMs can be classified as (i) alkaloids and nitrogen-containing compounds

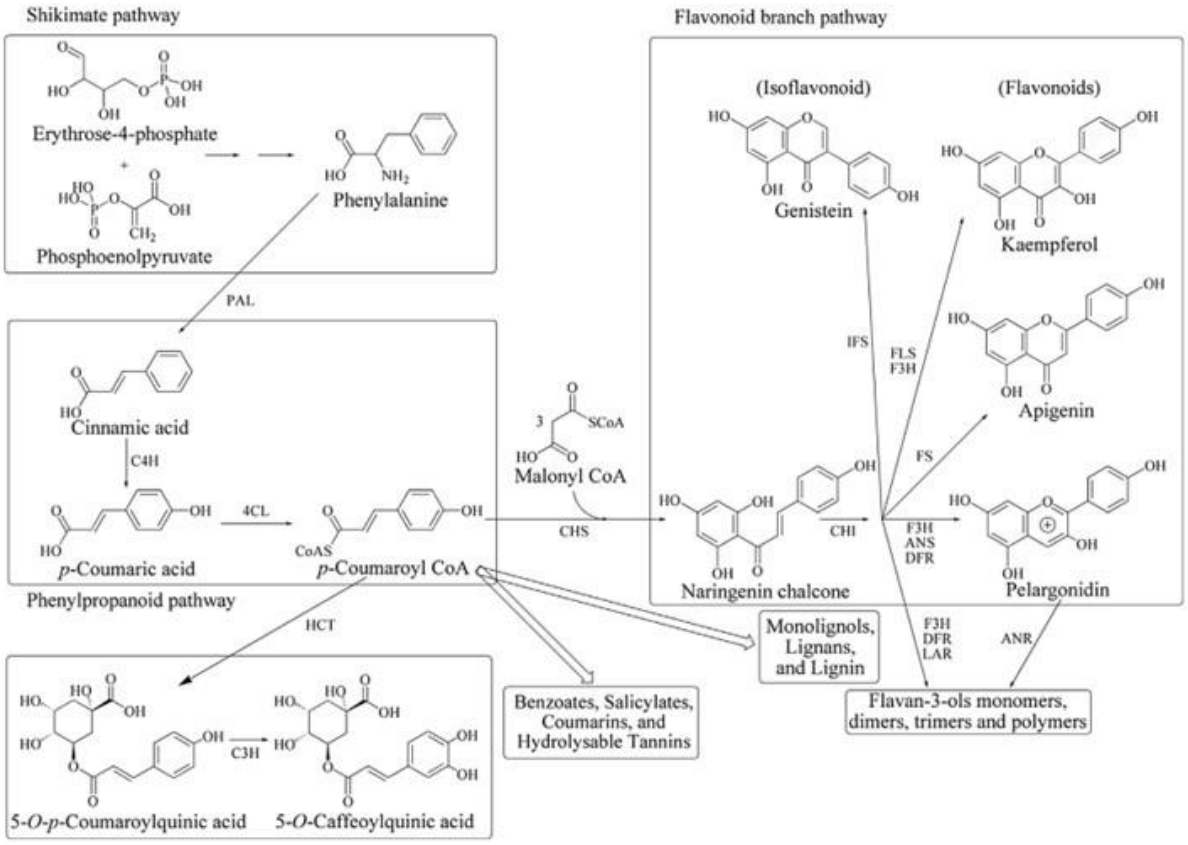

Figure 2. Schematic of the major branch pathways of (poly)phenol biosynthesis. PAL, phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaroyl:CoA-ligase;HCT, hydroxycinnamoyl transferase; C3H,p-coumarate-3-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase; ANS, anthocyanidin synthase; DFR, dihydroflavonolreductase; FS,flavone synthase; FLS,flavonol synthase; F3H,flavanone 3-hydroxylase; IFS, isoflavone synthase; ANR, anthocyanidin reductase; LAR, leucoanthocyanidin reductase. From Cheynier et al. 2013

(e.g. cyanogenic glycosides, alkaloids, and glucosinolates), (ii) phenolic compounds or phenylpropanoids (e.g. flavonols and tannins)(Figure 2), and (iii) terpenes (e.g. isoprenoids) (Wink, 2015; Ashraf et al., 2018; Patel et al., 2020). SMs present core structures (i.e. aglycones) comprising relatively few carbon atoms (e.g. 15 C for flavonoids and 9 C for cinnamic acids). The large structural diversity observed among these metabolic classes arises from different decorations commonly added to the core structures. Among these, glycosylation, i.e. conjugation to a sugar moiety, acylation, i.e. addition of an acyl group, and hydroxylation, i.e. addition of a hydroxyl group, give a substantial contribution to their diversity and impact their biological activity,

localisation, transport and storage (Louveau and Osbourn, 2019; Alseekh et al., 2020; Boutet et al., 2022). Glycosylation of phenylpropanoids is catalysed by uridine diphosphate (UDP)-dependent glycosyltransferases (UGTs) (Louveau and Osbourn, 2019), while phenylpropanoid acylation and hydroxylation is catalysed by acyltransferases and hydroxylases, respectively (Alseekh et al., 2020).

A given SM decoration is specific to a phylogenetically restricted groups of species, developmental phase, tissue or cell type, or to favourable or unfavourable environmental conditions (Howe and Jander, 2008; Ashraf et al., 2018). Hence, the regulation of SM synthesis and decoration contributes much to plant adaptation and survival in a wide range of habitats. Plant domestication, a process of natural and intentional evolution accomplished via either trait selection or cultivation management, was undoubtedly a process that inadvertently modified crops original metabolome (Dar et al., 2021). Although plant genotype is important to determine SMs diversity, it should also be considered that a central role is also played by the environmental conditions to which plants are subjected (Ballhorn et al., 2011; Patel et al., 2020). In fact, plants produce SMs in response to numerous environmental stressors, including drought (Kleinwächter and Selmar, 2015; Slama et al., 2015). Mevalonic acid, shikimic acid, phenyl propanoic acid, and methylerythritol phosphate are examples of SM pathways that are affected by water availability (Akhi and Sanaullah Biswas, 200AD; Banerjee and Sharkey, 2014). Drought causes oxidative stress in plants, resulting in reactive oxygen species (ROS) production that cause several cell damages. SMs scavenge ROS to neutralise free radicals and protect plant cells and enzymes (Mierziak et al., 2014; Treml and Šmejkal, 2016). Under stress conditions, the overaccumulation of flavonoids lead an enhancement of oxidative and drought tolerance (Nakabayashi et al., 2014). Moreover, under drought conditions, another metabolic category present in higher concentrations are terpenoids (Jogawat et al., 2021). SMs accumulation improves stress tolerance in plants by modifying physiological and biochemical factors (Kabera et al., 2014). This means that plant tolerance to water stress can be monitored thanks to the analysis of its metabolome (Arbona et al., 2013; Kumar et al., 2021).

Several studies investigated the relationship between water stress and metabolomic modifications on horticultural crops, such as tomato (Toscano et al., 2019; Hou et al., 2020). A study on grape showed that an irrigation reduction of 70% of the Etc, the levels of metabolites such as cinnamic acid, naringenin chalcone, naringenin, and eriodyctiol increased significantly (Yang et al., 2020). On olive the application of reduced deficit irrigation led to an increase in fruit squalene amount, a compound belonging to the category of terpenoids (Martinelli et al., 2012). In fact, terpenoids have an important role in oxidative stress reduction, preventing membrane and protein disintegration (Akhi and Sanaullah Biswas, 200AD).

AIM OF THE STUDY

Based on what described so far, it is clear that deficit irrigation is an interesting strategy for water savings and fruit quality optimization. However, results currently available usually are focused to a limited number of physiological parameters in each study, making more difficult to build a strong general picture on the basis of the conclusions obtained. Moreover, the effects of deficit irrigation on fruit quality are sometimes contradicting and thus there is the need to continue the researches in this sense.

The objective of this study is to evaluate the effect of deficit irrigation on the plant physiological performance and fruit quality development of different fruit crops. In particular the following aspects were considered:

- Water potentials and leaf gas exchanges.
- Fruit development.
- Vascular flows to/from the fruit.
- Sap flow.
- Fruit quality.
- Fruit metabolome.

The studies presented here focus on three fruit crops: apple and apricot, whose cultivation is spread worldwide and sour cherry, typically considered a minor fruit crop but increasingly studied for its benefits on human health.

The results of this study will add value and knowledge to the responses of these physiological processes on fruit crop, whether they may be enhanced or reduced, and on whether a particular irrigation management is more suitable than others to improve fruit and nutraceutical quality. Also, these findings would be a further supplement to the pool of information concerning fruit crop behavior influenced by deficit irrigation.

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CHAPTER II

APPLE FRUIT VASCULAR FLOWS UNDER DEFICIT IRRIGATION

ABSTRACT

Due to climate change and increasing water scarcity, apple production is threatened in some typical productive regions since the traditional water requirements are difficult to be met. In this context, it is extremely important to understand which are the plant and fruit physiological responses under a reduced irrigation management throughout the growing season. The trial was performed in 2020 and 2021 in the Po Valley, Italy in an apple orchard, cv. Gala. The irrigation treatments imposed were: 50% and 100% of the crop evapotranspiration rate. The main plant physiological parameters such as fruit growth, water potentials and leaf gas exchanges were monitored throughout the seasons until harvest. Fruit gauges were used to measure apple vascular fluxes in control and stressed trees. During both seasons, shoot and fruit were able to maintain similar growth patterns between treatments. Leaf water potential was affected only in 2020 and only in the early morning, while stem water potential was the only "water status" parameter negatively affected by deficit irrigation. Beside stem water potential, 50% irrigated trees occasionally decreased also photosynthesis during the first year, but in 2021 no difference was recorded between treatments. This different physiological pattern suggests the hypothesis of a sort of "drought stress memory" acting to avoid a decrease in plant physiological performance. Daily pattern of fruit vascular flows presented different trends depending on the irrigation treatment applied, however no differences were found on the cumulative flows. Fruit weight and soluble solid content responded to stress with a slight decrease in fruit size and an increase in sweetness in 50% irrigated fruit, while flesh firmness seemed to be not affected. Fruit total phenolic content was not influenced by reduced irrigation, probably because the stress imposed was not sufficient to activate an up-regulation in the synthesis of specialized metabolites.

INTRODUCTION

According to FAOSTAT in 2020 Italy was the 6th country in terms of total apple production, after China, USA, Turkey, Poland and India with a total of more than 2.46 million of tons of yearly production (FAOSTAT). At national level, apple represent the second most important crop in terms of production, following only grapevine. However, water scarcity represents an important issue threatening apple cultivation worldwide. Lack of water weakens the plant in the short and long-term, making it more susceptible to biotic and abiotic stresses. In this context, the application of Regulated Deficit Irrigation (RDI) can be an alternative tool to save water, while maintaining the orchard productivity. Although the idea of RDI was initially put forward with the intention of regulating the amount of vegetative growth in peach (Chalmers et al., 1981; Mitchell and Chalmers, 1982), now the main purpose for its application is to increase orchard water use efficiency. Several studies demonstrated that plants such as apple (Chenafi et al., 2016; Valverdi et al., 2020), peach (Falagán et al., 2016; Girona et al., 2003; ZHOU et al., 2017), apricot (Pérez-Pastor et al., 2009, 2007; Torrecillas et al., 2018), citrus (Gasque et al., 2016; Grilo et al., 2019; Saitta et al., 2021) and grapevine (Brouziyne et al., 2018; Pagay, 2016) can favorably respond to RDI application. However, other studies demonstrated that RDI can decrease yield and fruit size in apple, regardless of the timing of application (Landsberg and Jones 1981; Ebel et al. 2001; Mpelasoka et al. 2001). To identify the most suitable periods to apply deficit irrigation (DI), it is important to monitor the most sensitive plant indicators. For example, it is well-know how stem water potential is a key physiological indicator for plant water status (McCutchan and Shackel, 1992). However, in recent years Boini et al. (2019) found that daily fruit growth can be a potential effective indicator to recognize the onset of mild stress in apple trees.

Fruit daily growth patterns are frequently characterized by periods of shrinkage and swelling, ending with a permanent increase in fruit size after a period of 24 hours (Fishman and Génard, 1998; Lang, 1990; Morandi et al., 2011a, 2012). In apple, seasonal fruit growth is represented by an expo-linear model, characterized by an early stage (cell division) of increasing growth rates, followed by a second stage of constant growth rates (cell expansion) that lasts until harvest (Lakso et al., 1995). Different stages of fruit development result in distinct daily growth patterns. During

the cell division stage xylem water may flow backwards from the fruit to the stem during the midday hours, when leaf transpiration losses are at their maximum and stem water potential becomes more negative than the fruit. This dictates times of contraction throughout the day, followed by periods of fast rehydration in the late afternoon and the night, when leaves decrease stomatal conductance and increase their water potentials (Lang, 1990; Morandi et al., 2011b). During fruit development the xylem progressively loses its functionality (Drazeta, 2004). For this reason, phloem flow is crucial for fruit growth during cell expansion, when xylem vessels become less efficient. At this stage the fruit grows steadily throughout the day, albeit at a slower rate in the middle of the day (Lang, 1990). In spite of this, water potential gradients between the stem and the fruit are clear drivers of vascular flows to and from the fruit, therefore stem water potential has a significant impact on fruit growth regardless of the phenological stage. Therefore, when trees are subjected to drought stress, their stem water potential decreases, making it more challenging for the fruit to attract water from the vascular tissue, resulting in slower fruit growth rates. Strong correlations between seasonal average stem water potential and final fruit size provide evidence of this effect (Naor et al., 1995, 1997, 2008). In this context, it is still unclear how the daily patterns of fruit vascular flows respond to deficit irrigation in apple and their consequences on fruit development.

DI has been shown to hasten the ripening of fruit, increase the firmness of the flesh, as well as total soluble solids (TSS) and aroma volatiles, both during the ripening phase and after the fruit storage (Mpelasoka et al. 2001; Mpelasoka and Behboudian 2002). Deficit irrigation might also result in a reduced apple firmness, according to literature, which is linked to the anticipated maturity of fruit under water stress (Drake et al., 1981; Mills et al., 1994). Other research, however, has revealed that apples from non-irrigated plots were firmer than those from irrigated plots because fruit from non-treated plots had smaller size (Assaf et al., 1975).

It is known how water stress affects apple tree physiological responses, however there are no information available on how fruit vascular flows are affected on a daily scale and which are the possible repercussion on final fruit quality. The objective of this study is thus to assess the diurnal response of fruit vascular flows under deficit

irrigation, monitoring as well the effects on plant eco-physiological performance and harvest fruit quality.

MATERIALS AND METHODS

Plant material and trial set up.

The trial was conducted during the vegetative seasons 2020 and 2021 at the experimental farm of the University of Bologna located in Cadriano, Bologna, Italy (44°32'53"N 11°24'45"E). The trial was set in an *Imperial Gala* apple orchard with trees grafted on M9 and trained at solaxe (Lauri and Lespinasse, 1998). The orchard density was 3800 trees ha^{-1} with plants spaced 1 m x 3.8 m. Two irrigation treatments were imposed: 50% and 100%, corresponding to the 50% and 100% respectively of the crop evapotranspiration rate. Irrigation needs were scheduled based on the regional decision support system platform "Irriframe" (www.irriframe.it), provided by Consorzio per il Canale Emiliano Romagnolo (CER) whose irrigation advice is based on the soil water balance. These treatments started at 64 (2020) and 39 (2021) days after full bloom (DAFB) and lasted until harvest, for a total of 48 and 70 days under deficit irrigation in 2020 and 2021, respectively.
In 2020, a total of 6 plants per treatment were selected, divided in two blocks. In 2021, a total of 9 plants per treatment, divided in 3 blocks were selected for measurements. Control plants were irrigated using a drip irrigation system with 2.5 drippers per tree (the distance between emitters was 0.4 m) with a flow of 2.0 L h^{-1} per dripper. Reduced irrigation was applied by using drippers with half the flow of the control ones (1.0 L h^{-1} vs. 2 L h^{-1}) This means that per each irrigation event the hours of irrigation were the same for both treatments. In 2020 plants received 143 mm and 74 mm in 100% and 50% treatments, respectively, while in 2021 irrigation was 268 mm in 100% and 135 mm in 50% irrigated plants. Temperature, relative humidity and rainfall data were collected every 15 min from a weather station (Wi-Net s.r.l. Cesena, Italy). With the collected data, vapour pressure deficit (VPD), was calculated according to the equation reported by Higgs and Jones (1982).

Full bloom occurred on April 15th, 2020, and on April 9th, 2021, while fruit were harvested on August 5th and August 6th in 2020 and 2021, respectively. The total duration of the fruiting cycle corresponded to 112 days in 2020 and to 119 days in 2021.

Measurements

Fruit and shoot growth

During both 2020 and 2021 seasons, 72 fruit and 72 shoots per treatment were monitored at regular times interval during the season. Shoot length was monitored using a measuring tape, while fruit diameter was moniterd using a digital caliper (Mitutoyo,Kanagawa, Japan) equipped with a datalogger (hkconsulting.it, last access:26-01-2023) . For each fruit, diameter (D) data (mm) were converted to weight (W) (g), by the following conversion equation, stemming from more than 300 Gala apples (R^2>0.99) (Boini et al., 2019) (Eq. 1):

$$W(g) = a \cdot D\,(mm)\text{^}b \qquad Eq.1$$

where a and b were 0,0006 and 2,909, respectively.

Fruit and shoot absolute growth rates (AGR) (g $fruit^{-1}$) were calculated at each recording time (t), using the following equation (Eq. 2):

$$AGR_{t1} = \frac{FW_{t1} - FW_{t0}}{t_1 - t_0} \qquad Eq.2$$

where FW_{t1} was the fruit weight at recording time t1 and FW_{t0} was the fruit weight at the previous recording time.

Water relations

Midday stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials were monitored at 58, 92 and 106 DAFB in 2020 and at 47, 82, 104, 112 and 116 DAFB in 2021, on at least 4 trees for each treatment, using a Scholander (Soilmosture Equipment Corp. Santa Barbara,

U.S.A.) pressure chamber. Full-day measurements were performed with data collected at 04:00, 9:30, 12:00 and 15:00h in all the measurements dates in 2020 and at 47, 82 and 116 DAFB in 2021. Ψ_{leaf} were measured on one well exposed shoot leaf per tree following the methodology proposed by Turner and Long (Turner and Long, 1980). Ψ_{stem} were 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 aluminum foil at least 90 minutes before the measurement to allow equilibration with the stem, according to the methodology described by McCutchan and Shackel (McCutchan and Shackel, 1992) and by Naor et al. (Naor et al., 1995).

Leaf gas exchanges

Leaf gas exchanges were measured on the same days and timings (except at 4:00h) of the water potential measurements, using an open circuit leaf gas exchange system equipped with a LED light source (Li-COR 6400, LI-COR, Lincoln, Nebraska, USA). Measurements were carried out on the same plants, choosing one fully illuminated and developed leaf. During each measurement, light intensity was maintained constant at the natural photosynthetically active radiation (PAR) level experienced by the leaves immediately before the measurements, and the CO2 concentration was set at 400 ppm. The main parameters considered in the data elaboration were net photosynthesis, transpiration, and stomatal conductance (gs).

Xylem, phloem and transpiration flows

From 100 DAFB to 110 DAFB both in 2020 and 2021, the fruit vascular and transpiration flows of both treatments were assessed, following the methods reported by Morandi et al., 2011; 2012 and adapted from Lang (1990). Diameter variations over time were simultaneously measured on 12 well-exposed fruit planted on 3 trees, on both sides of the canopy. At least three fruits on each tree were either "intact" (had normal vascular connections), "girdled" (having the phloem link disrupted), or "detached" (with all vascular connections severed). The xylem and phloem fluxes were then calculated as a difference between fruit in different conditions. Continuous fruit diameter variations were monitored using custom built gauges (Morandi et al., 2007) connected to a wireless data-logging system at intervals

of 15 minutes (Winet srl, Cesena, Italy). The gauges were made of a light, stainless steel frame that held a transducer with changeable linear resistance (Megatron Elektronik AG & Co., Munich, Germany). The relative changes of fruit fresh weight in each time interval (t) were then calculated for each fruit: normal (N), girdled (G) and detached (D); xylem (X), phloem (P) and transpiration (T) flows were computed using the following equations (Eq. 3, 4, 5):

$$P_t = N_t - G_t \qquad Eq.3$$

$$X_t = G_t - D_t \qquad Eq.4$$

$$T_t = D_t \qquad Eq.5$$

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}).
This approach *assumes* that girdling and detachment have no impact on xylem flow or transpiration rate. For the xylem, this assumption has been partly confirmed by magnetic resonance measurements, showing no changes in xylem flows along intact and girdled stems (van de Wal et al., 2017). However, the approach can lead to some inaccuracies at specific times during the day, which can result in under- or over-estimations of phloem and xylem flows (Lescourret et al., 2001). To now, this is the only method that enables calculation of vascular and transpiration flows on a statistically sound number of samples and in a field-short time scale.

Fruit quality and Total Phenolic Content (TPC)
Standard fruit quality parameters were measured on 3 replicates per treatment, each with 20 fruit. Fruit caliber and weight, flesh firmness and soluble solid content were measured with a digital caliper (Mitutoyo,Kanagawa, Japan) , a digital weighting scale (Kern), a fruit texure analyzer (FTA, by Güss) and a digital refractometer (Digital handheld PAL-1, ATAGO), respectively. In addition, during each year, fruit were sampled for TPC analysis at two times during the season: at 92 and 112 DAFB in 2020 and at 106 and 118 DAFB in 2021. A total of 12 fruit per treatment were collected from 3 different plants. Then, samples were immediately brought to the laboratory,

the skin and the pulp separated and freezed with liquid nitrogen and stored in a freezer at -80°C. Later, the TPC was evaluated according to the Folin-Ciocalteau method proposed by Cantin et al. (Cantín et al., 2009). In brief, 5 g of dried material were homogenized with an Ultraturrex for 2 minutes on ice, with 10 mL of extraction solution, consisting of 0.5N HCl in methanol/Milli-Qwater (80%v/v). The mixture was then incubated overnight at 4 °C and the following day centrifuged for 20 min at 4 °C and 20000g. Supernatant was recovered, and the volume measured. The total phenolic content was determined according to the Folin-Ciocalteu method (Singleton et al., 1965). The method consisted of mixing 500 µL of the extract diluted in water with 500 µL of Folin-Ciocalteu's reagent. After 3min of reaction, 1mL of 1N sodium carbonate (Na2CO3) was added. The tubes were mixed for 15 s and then put in the dark for 60 min at 20 °C. Absorbance was measured at 725 nm using a spectrophotometer (Biochrom). The standard calibration curves were daily prepared using gallic acid (3,4,5-trihydroxy- benzoic acid). The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of FW.

Statistical analysis

For each treatment, averages and standard errors (SE) were determined for each measurement time, for all the parameters considered. A Student's t-test was used to compare treatments for each date of assessment. Data elaboration and statistical tests were performed using Excel.

RESULTS

Meteorological data

In 2020, the total precipitation amount occurred during the trial was 93 mm. The highest temperature recorded in the orchard was 38.4°C at 108 DAFB. In 2021, total precipitations were 40 mm while maximum temperature was 37.3°C, at 87 DAFB (Figure 1). The highest VPDs recorded were 3.7 kPa and 4.9 kPa in 2020 and 2021, respectively (Figure 1).

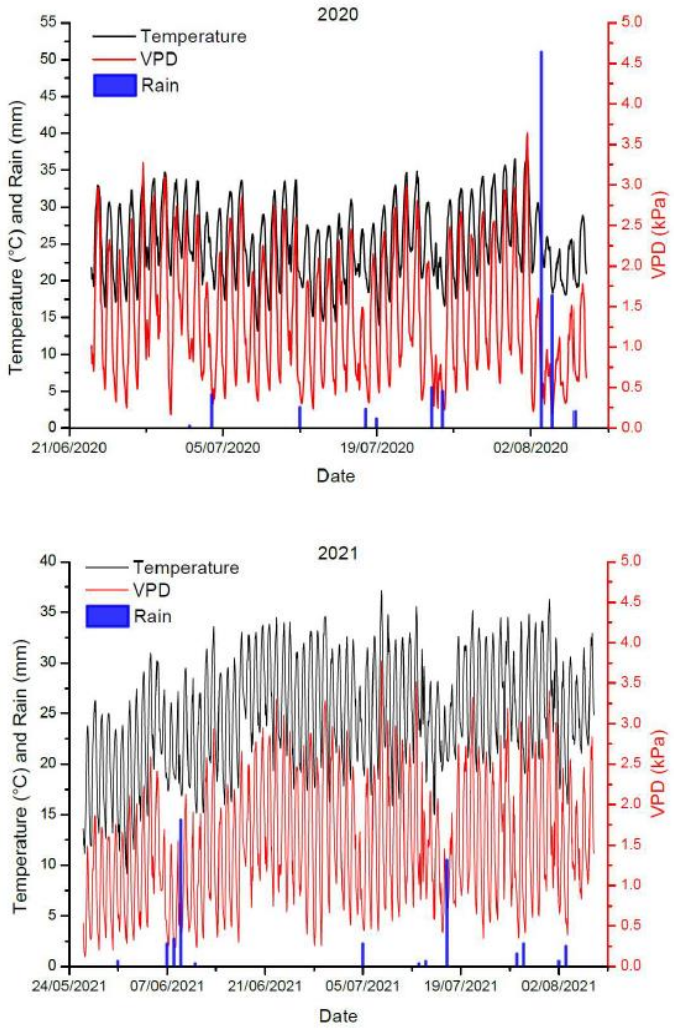

Figure 1. Seasonal air temperature (black line), VPD (red line) and precipitation (blue bar) in 2020 and 2021. Each graph displays weather conditions from the beginning of the RDI treatments, at 69 DAFB and 47 DAFB in 2020 and 2021, respectively.

Fruit and shoot growth

Apple fruit growth showed a linear growth pattern in 2021 (Figure 2a). Fruit from plants receiving different amount of water were able to maintain similar growth rates, reaching values around 150 g at harvest. Fruit from both treatments showed a slight decrease in absolute growth rate (AGR) at 82 and 96 DAFB, then AGR increased again at 104 DAFB to 4.3 and 3.7 g d^{-1} in 50% and control respectively (Figure 2b). No statistically significant differences were recorded along the whole season.

Seasonal shoot growth showed a similar trend in the two treatments, with no differences in shoot length along the season (Figure 3a). The highest shoot AGRs were recorded at 82 DAFB, then, both treatments started to decrease their AGRs. The 50%

treatment decreased its AGR to 0.075 cm day^{-1} at 104 DAFB but afterwards at 109 DAFB increased again to 0.35 cm day^{-1}. (Figure 3b).

In 2020 as well fruit and shoot growth were monitored weekly, but when analyzing the results it was noticed that the samples selected showed statistical significant differences before the beginning of the application of RDI. Since this could lead to the misinterpretation of the results, these data have not been considered and will not be presented. For this reason, in figures 2 and 3 are shown only data collected in 2021.

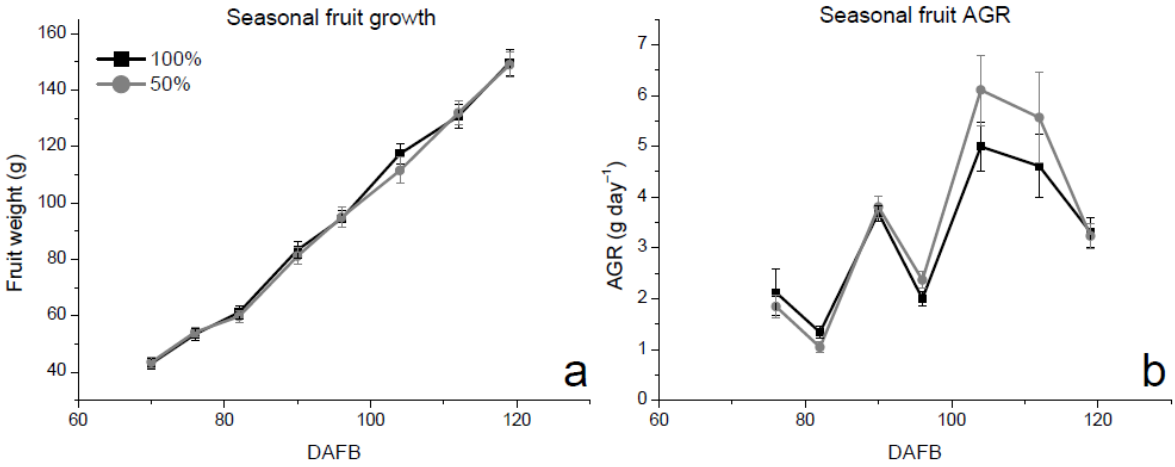

Figure 2. Seasonal fruit growth (+ SE) (a) and fruit absolute growth rate (AGR) (b) for 100% (black line) and 50% (grey line) treatments. Diameter values were converted to mass using Eq. 1. Each point represents the mean of 72 fruit. SE bars smaller than symbols are not visible. Stars represent statistical differences for Student's t test $p < 0,05$.

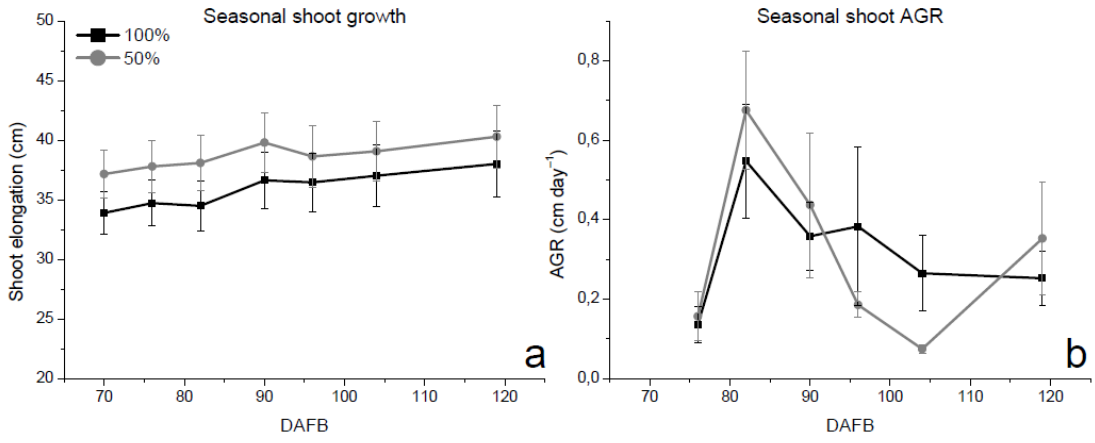

Figure 3. Seasonal shoot growth (+ SE) (a) and shoot absolute growth rate (AGR) (b) of 100% (black line) and 50% (grey line) in 2021. Each point represents the mean of 72 shoots. Stars represent statistical differences for Student's t test $p < 0,05$.

Stem and leaf water potentials

Stem (Ψ_{stem}) and leaf (Ψ_{leaf}) midday water potentials tended to decrease throughout the season. Reduced irrigation induced a decrease in Ψ_{stem} at 92 DAFB in 2020 and at 82 and 112 DAFB in 2021 (Figure 4). At 112 DAFB in 2021 (Figure 4b) the 50% irrigation treatment decreased Ψ_{stem} to -1.5 MPa while the 100% treatment was -1.2 MPa. Leaf water potential did not show any differences in the midday values along the season.

On a daily scale, in 2020 a decrease in stem water potential of 50% vs. 100% was detected at 9, 12 and 15 h, at 92 DAFB (Figure 5c). In addition, also leaf water potential was affected by the irrigation treatment applied, showing lower values in 50% plants at 9 h both at 92 and 106 DAFB 2020. In 2021, the reduced irrigation slightly affected 50% plants, showing a decrease in Ψ_{stem} only at 9 h at 82 DAFB and at 15 h at 116 DAFB.

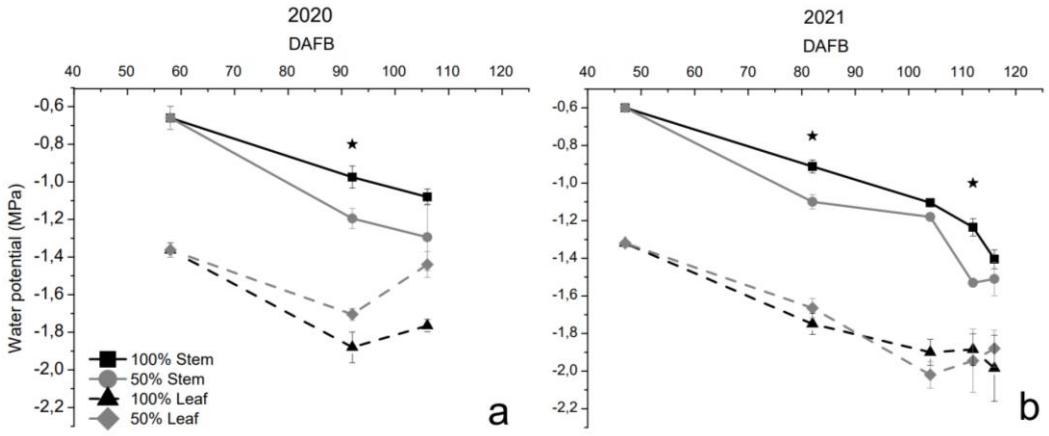

Figure 4. Seasonal midday stem and leaf water potentials (+ SE) of 100% (black lines) and 50% (grey lines), measured in 2020 (a) and 2021 (b). Each point represents the mean of 4-6 replicates. Stars represent statistical differences for Student's t test $p < 0,05$.

Seasonal leaf gas exchanges

During both seasons leaf photosynthesis showed a variable pattern with an initial decrease followed by an increase at 96 and 82 DAFB in 2020 and 2021, respectively

(Figure 6a and 6b). At 106 DAFB, in 2020, plants subjected to 50% RDI showed a lower photosynthesis with values around 7.5 μmol m^{-2} s^{-1}, while control leaves showed values of 18.3 μmol m^{-2} s^{-1}.

Stomatal conductance showed different patterns in the two years, with a high variability in the absolute values during the season. Differences among treatments were detected only once, at 106 DAFB in 2020, when RDI trees showed a reduction in stomatal conductance compared to controls, with values of 0,07 and 0,23 mol m^{-2} s^{-1} in 50% and 100% trees, respectively (Figure 6c). In 2021, trees showed a similar pattern regardless of the treatment, however values were lower than the ones showed during the previous year, never being above 0,25 mol m^{-2} s^{-1} (Figure 6d).

In 2020, the pattern of transpiration followed the one of stomatal conductance, with lower values at 106 DAFB (Figure 6e). In 2021, a more variable pattern was recorded between 82 DAFB and 106 DAFB, for both treatments, with values ranging from 6,5 and 6,0 to 1,9 and 5,9 mol m^{-2} s^{-1} in 100% and 50% treatments, respectively. At the end of the season, controls showed lower values than RDI trees (Figure 6f).

Fruit vascular flows

The daily flows from/to the fruit in the period considered (106-108 DAFB in 2020 and 104-110 DAFB in 2022) did not show any significant difference between irrigation treatments. However, it can be noted how the fluxes varied in the two years considered. In fact, fruit growth was lower in 2020 than in 2021 (Figure 7a and 7b) while the relative transpiration rate was higher in 2021 compared to the previous year (Figure 7c and 7d). Xylem and phloem flows maintained a similar pattern in the two years (Figures 7e, f, g, h). Regarding the daily patterns of fruit growth and vascular flows, several parameters were different between treatments in both years (Figure 8). In 2020, fruit growth showed a marked shrinkage during the midday hours, reaching values of -0.04 mg $g^{-1}min^{-1}$ and -0.01 mg $g^{-1}min^{-1}$ in 100% and 50%, respectively (Figure 8a). In the late evening and at night, 50% irrigated fruit showed the lowest values, with significant decreases compared to controls. In 2021, the fruit shrinkage was not so evident as in 2020, although around midday and the first part of the afternoon the fruit stopped growing (Figure 8b). Transpiration presented different patterns between the years: in 2020 specific transpiration rate was higher than in 2021, with lower values in 50% irrigated fruit during most of the day (Figure

8c). In 2021, differences between treatments were less evident, however 50% fruit were able to continue transpiring also in the late evening (Figure 8d).

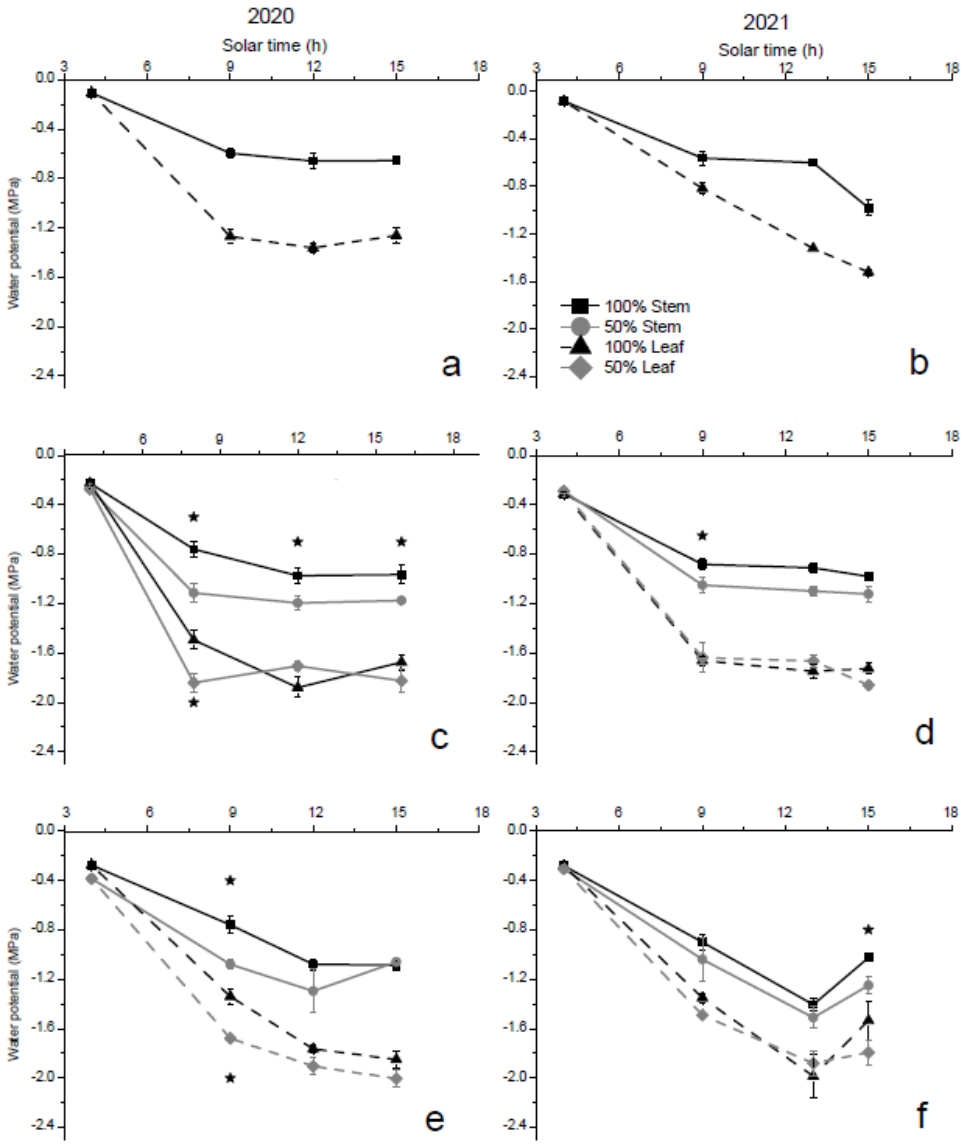

Figure 5. Daily patterns of stem (solid lines) and leaf (dash lines) water potentials (+ SE), in 50% (grey lines) and 100% (black lines) treatments. Measurements were performed at 58 (a), 96 (c) and 106 (e) DAFB in 2020 and at 47 (b), 82 (d) and 116 (f) DAFB in 2021. Each point represents the mean of 4-6 replicates. SE bars smaller than symbols are not visible.

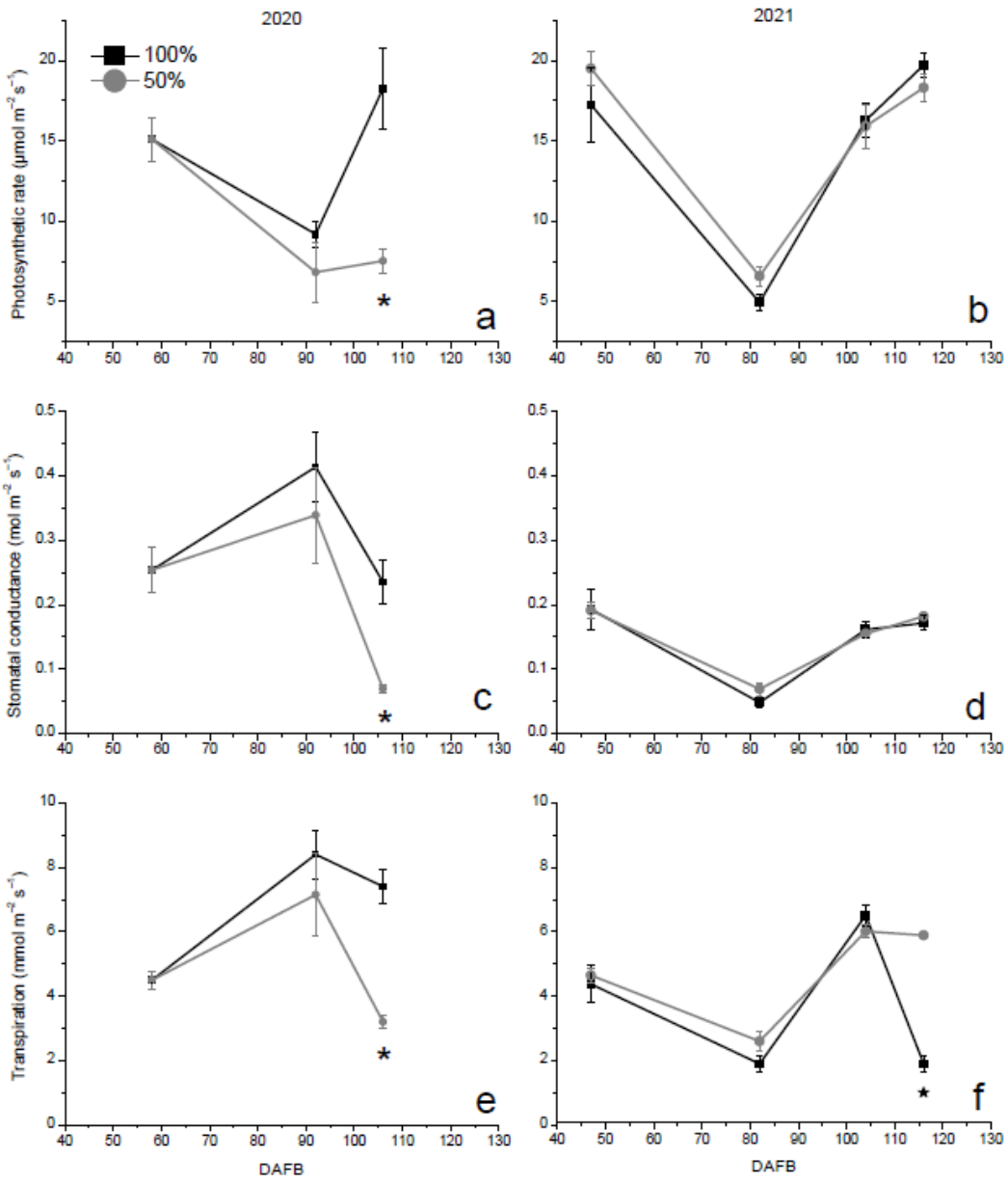

Figure 6. Seasonal leaf net photosynthesis (+ SE) (a,b), leaf transpiration (+ SE) (c, d) and stomatal conductance (g_s) (+ SE) (e, f) in 100% (black lines) and 50% (grey lines) treatments. Leaves were measured at 58 (a), 96 (c) and 106 (e) DAFB in 2020 and at 47 (b), 82 (d) and 116 (f) DAFB in 2021. Each point represents the mean of 4-6 replicates. Stars represent statistical differences for Student's t test $p < 0,05$.

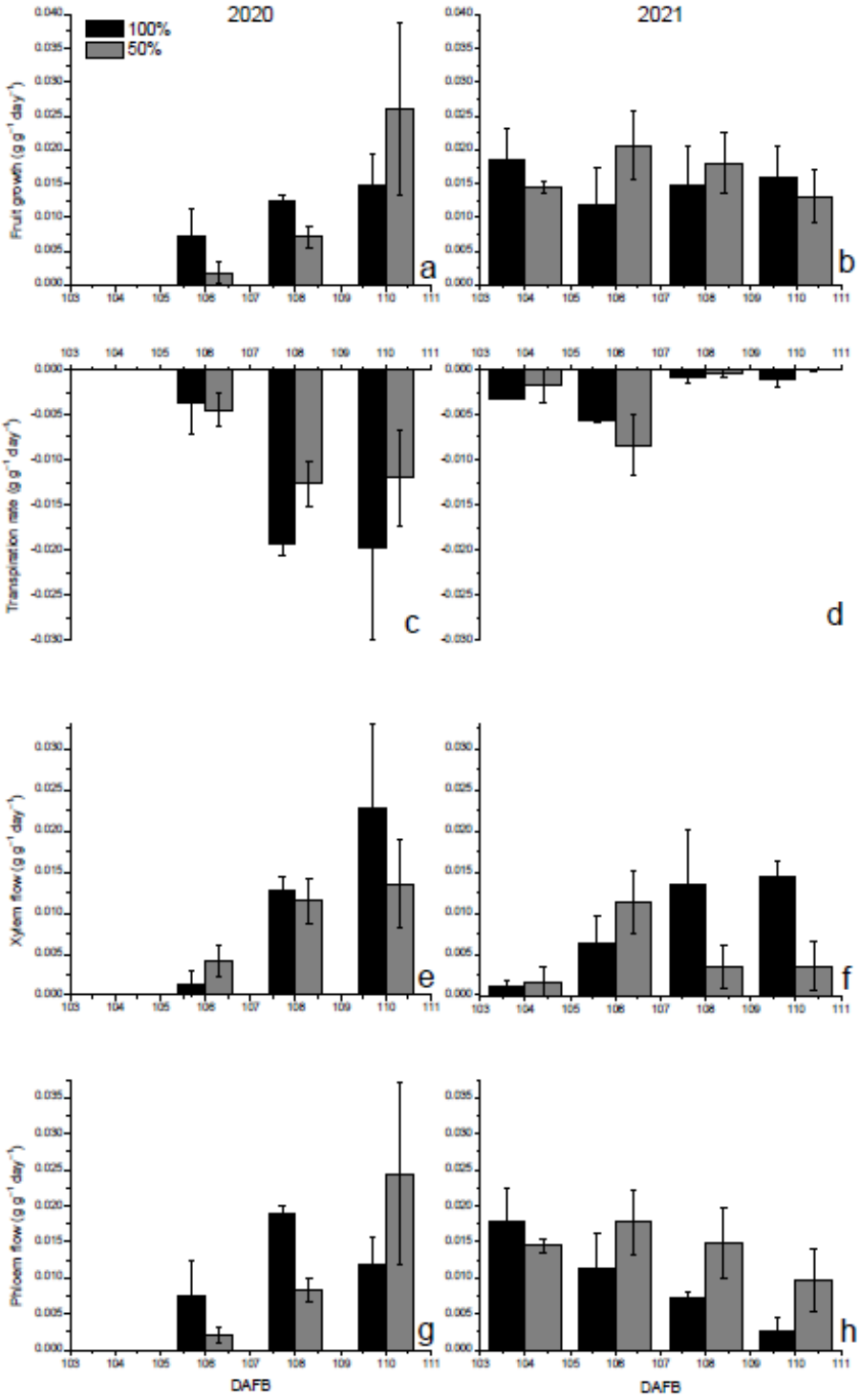

Figure 7. Mean (+ SE) of the relative growth rates (RGR) (a, b), transpiration (c, d), xylem flow (e, f), phloem flow (g, h) at 104, 106 and 108 DAFB in 2020 and at 104, 106, 108 and 110 in 2021 in 100% (black bar) and 50% (grey bar).

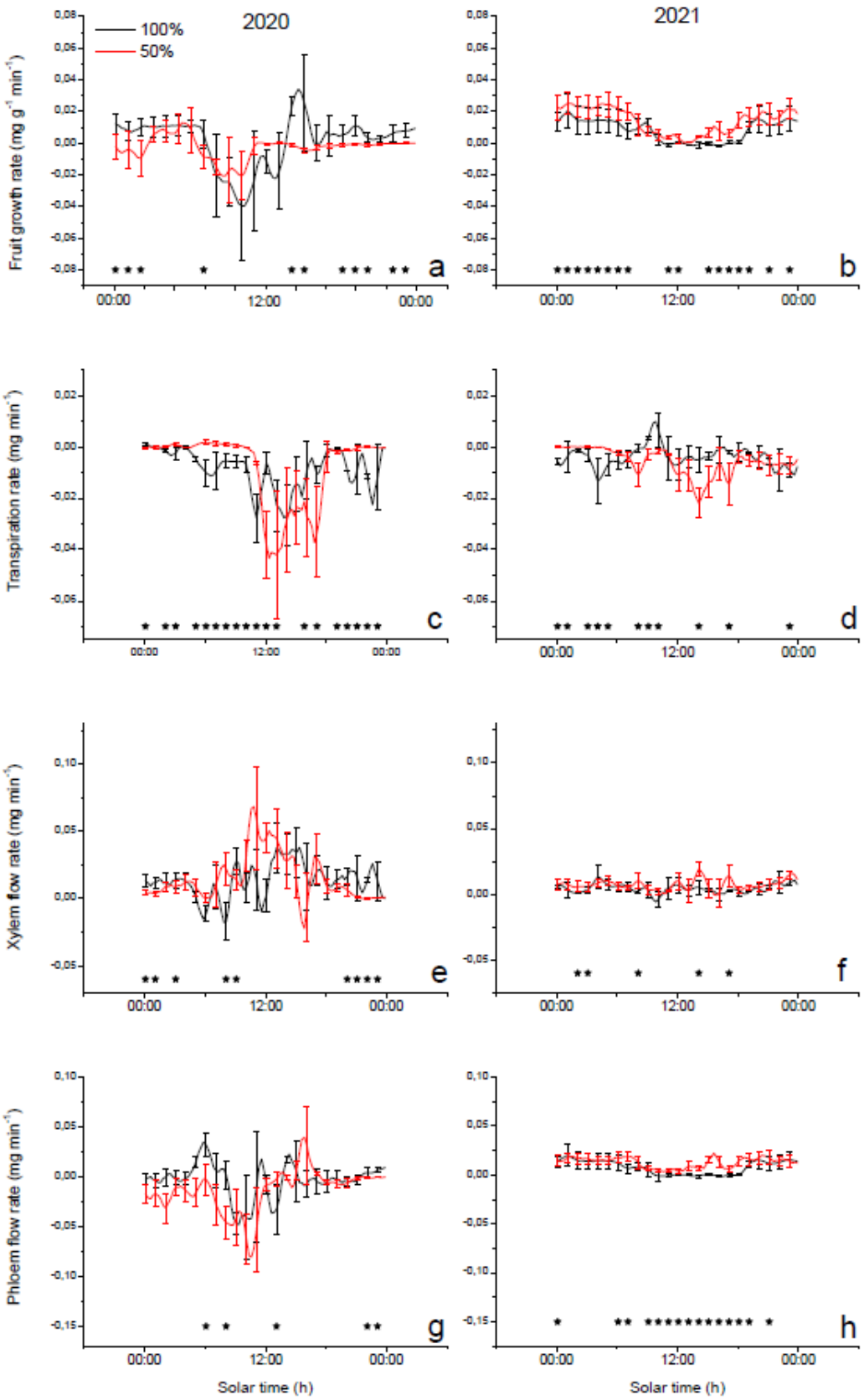

Figure 8. Average diurnal courses of fruit relative growth rate (RGR) (a, b) and specific transpiration (c, d), xylem (e, f), phloem (g, h) flow rates (mg $g^{-1}min^{-1}$) at 107 DAFB both in 2020 and 2021 in 100% (black line) and 50% (grey line).

The highest xylem inflow in 50% fruit was recorded in 2020 during the middle part of the day, whereas in 2021 50% fruit the higher xylem inflow happened during the night, recording a xylem backflow in the early afternoon (Figure 8e, 8f).
Phloem inflow presented higher values in 100% fruit during the late morning while 50% showed higher values in the early afternoon in both years (Figure 8g, 8h).

Fruit quality and total phenolic content

Fruit size and weight showed a decrease in both years (Table 1). However, an increase in the SSC content in 50% fruit was observed in 2021 (Table 1). Fruit firmness instead showed similar values regardless of the irrigation treatment applied. Total phenolic content did not show any difference between treatments for all the measurements carried out except at 112 DAFB 2021, one week before harvest, when 50% irrigated fruit showed values of 0.85 mg of GAE g^{-1} of frozen tissue against 0.77 mg of GAE g^{-1} of frozen tissue of controls. However, this difference disappeared at harvest.

Table 1. Apple fruit quality at harvest in 2020 and 2021. Fruit caliber, weight, flesh firmness and soluble solids content were measured. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

	2020			
	Caliber (mm)	**Weight (g)**	**Flesh firmness (kg/cm^2)**	**SSC (°Birx)**
100%	73.4	175.2	8.5	11.2
50%	68.8	144.2	8.6	11.6
Sign.	*	*		

	2021			
	Caliber (mm)	**Weight (g)**	**Flesh firmness**	**SSC (°Birx)**
100%	72.4	162.7	9.7	11.1
50%	69.7	143.7	9.9	11.5
Sign.	*	*		*

Table 2. Total phenolic content in apple flesh and skin tissues, in 2020 and 2021. Values are expressed as mg of GAE g^{-1} of frozen tissue. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

		92 DAFB		112 DAFB	
		Skin	Flesh	Skin	Flesh
	100%	4.26	0.13	4.09	0.29
2020	50%	4.47	0.14	3.88	0.23
	Sign.				
		112 DAFB		119 DAFB	
	100%	1.63	0.75	0.77	0.17
2021	50%	1.33	0.88	0.58	0.13
	Sign.		*		

DISCUSSION

The two seasons considered in this trial were characterized by marked differences in the weather conditions, in terms of rain and temperature leading to different plant responses to deficit irrigation. As a matter of fact, 2020 was characterized by more than 90 mm of precipitation in the last 2 weeks before harvest which likely reduced the effect of the deficit irrigation treatment. In 2021, the average VPD was higher than in 2020 (Figure 1). Fruit growth in 2021 showed a linear pattern from 47 DAFB onwards, in accordance with what reported in literature for the cell expansion stage since the exponential stage typical of cell division usually happens earlier in the season (Lakso et al., 1995). Shoot and fruit were able to maintain similar growth patterns between treatments, thus indicating that trees were not affected by an irrigation reduction of 50% in their evapotranspiration needs (Figure 2 and 3). In this study, leaf water potential was affected only in 2020 and only in the early morning, while stem water potential was the only "water status" parameter negatively affected by deficit irrigation, reaching values even below the fixed threshold of -1 MPa for mild stress in apple (Naor et al., 1995; Naor, 2012; Lopez et al., 2018)(Figure 4 and 5). However, despite a difference in stem water potential was recorded among the two treatments, the values reached by the 50% irrigated trees did not indicate the presence of stress except for an occasional mild stress occurred at 112 DAFB when

stem water potential reached -1.5 MPa. Beside stem water potential, 50% irrigated trees occasionally decreased also photosynthesis during the first year, but in 2021 no difference was recorded between treatments (Figure 6). Considering the leaf gas exchanges at 116 DAFB 2021, it can be noted that Pn and g_s presented similar values in both treatments, while transpiration seems to be not in agreement with those results. This can lead to the hypothesis that during the measurement there some technical problems can have been incurred.

In any case, high levels of photosynthesis may not always represent the most resource-efficient solution in terms of productivity. This is because strong competition with the growing shoots can prevent carbohydrates from being allocated to fruit sinks (Dejong, 1999). Water potential gradients between the stem and the fruit are responsible for driving vascular flows that sustain fruit growth (Fishman and Gènard, 1998). This study showed that fruit receiving reduced irrigation were able to maintain similar daily vascular flows when compared to control fruit. In spite of the likely cause-effect relationship between stem water potential and fruit vascular inflows, a positive linear relationship was discovered to exist between midday stem water potential and fruit daily growth (Boini et al., 2019). In fact, it was found that fruit growth of less than 1.2–1.3 g $fruit^{-1}$ day^{-1} can be an indicator of the beginning of drought stress and, consequently, the requirement to increase irrigation. This reference value does not change from around 80 DAFB onwards because the weight of apple fruit remains the same from the beginning of cell expansion until harvest (Lakso et al., 1995). Clearly, this value is referring to the particular conditions (in terms of orchard age, crop load, and nutritional status, etc.) of the orchard where the experiment was carried out, and as a result, it is possible for it to differ for other types of orchards and varieties. All considered, daily fruit growth never fell under the proposed stress threshold of 1.3 g suggested by Morandi et al. (2016). Gala apples are known to show disruptions in the fruit xylem vessels (Drazeta et al., 2004) after approximately 55 days after full bloom, which leads to losses in xylem functionality. Therefore, beginning at 70 DAFB and continuing beyond that point, the growth of apple fruit may become less dependent on stem water potential in comparison to leaf gas exchanges. In fact, during this time of the year, apple fruit growth is almost entirely supported by phloem flow (Lang, 1990). The measuring seasonal fruit

growth and fruit size at harvest showed different values in the irrigation treatments. In fact, looking at Figure 2 it can be noted that no differences in fruit weight was observed, while in Table 1 fruit weight was reported to be statistically significant lower in fruit from 50% irrigated plants. This can be due to the reduced sample set considered when measuring fruit quality (60 fruit per treatment against 72 per treatment when measuring seasonal fruit growth).

Regarding fruit quality parameters, flesh firmness seemed to not be affected by deficit irrigation, being in contrast either to those studies that found a decrease in firmness (Drake et al., 1981; Mills et al., 1994) and those that found an increase in firmness due to the smaller size (Assaf et al., 1975). On the contrary, soluble solid content responded to stress with an increase in sweetness in 50% irrigated fruit. This response is in accordance to literature (Pérez-Pastor et al., 2007b ; Pérez-Pastor et al., 2009 ; Cabral et al. 1995 ; Mpelasoka et al.2001c; Naor et al 2004; Venturi et al 2021; Leib et al. 2006; Lopez et al. 2011 ; Treeby et al. 2007; Velez et al. 2007 ; Du et al. 2008). Total phenolic content was higher in the fruit skin while it was lower in the flesh. This is in line with what already stated by Kanizadeh, who found that the distribution of phenolic differs significantly between cultivars as well as between tissues (Khanizadeh et al., 2008). In addition, TPC did not show so marked difference in the fruit whose plants received different irrigation volumes. This could be due to the fact that the drought induced was extremely mild as showed by the stem water potential values recorded. However, at 112 in 2021 flesh tissue showed an increase in TPC in stressed conditions. This response might suggest a positive effect of drought stress on the biosynthesis of polyphenols.

Besides, genetics have a significant influence in regulating the polyphenol composition of apples and other fruits (Khanizadeh et al., 2007) but probably the stress applied was not enough to significantly activate those genes.

CONCLUSION

This trial had the aim to investigate the diurnal response of fruit vascular flows under deficit irrigation, monitoring also the effects on plant physiology and harvest fruit quality. Results showed that reduced irrigation decreased stem water potentials as well as leaf gas exchanges towards the end of the season. Fruit vascular flows showed

that xylem dysfunctionality is highly dependent on the environmental conditions, meaning that management practices can be slightly adjusted from year to year in order to maximize fruit quality and storability. For example, calcium application could be planned also slightly later in season if weather conditions delayed fruit xylem dysfunctionality. The response of fruit weight to DI was not straightforward, however soluble solid content responded to stress with an increase in sweetness in 50% irrigated fruit, while flesh firmness seemed to be not affected. Fruit total phenolic content was not influenced by reduced irrigation, probably because the stress imposed was not sufficient to activate an up-regulation in the synthesis of specialized metabolites. We can conclude that the current evapotranspiration clalculation in apples may overestimate the amount of water required by the tree, whereas a slight deficit irrigation may be beneficial because it does not significantly reduce fruit size while improving fruit sweetness.

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CHAPTER III

THE EFFECTS OF PROGRESSIVE WATER STRESS ON PLANT PHYSIOLOGY AND FRUIT NUTRACEUTICAL QUALITY. A STUDY ON SOUR CHERRY.

ABSTRACT

Sour cherry (Prunus cerasus L.) fruit have been studied in recent years for their positive effects on human health. However, little is known about their physiological reactions to the variability in environmental conditions coupled with water scarcity. This study was conducted in 2020 and 2021 to assess the physiological effects induced by water deficit in the last weeks before harvest. Starting from 59 (2020) and 55 (2021) days after full bloom (DAFB), a set of trees completely stopped receiving irrigation, while the control trees were irrigated on the basis of crop evapotranspiration. During each vegetative season we monitored: i) stem and leaf water potentials; ii) leaf gas exchanges; iii) fruit and shoot growth; iv) fruit quality at harvest (2021); vi) fruit total phenolic content; v) fruit anthocyanin concentration and vii) fruit metabolomic landscape. Data showed no differences in the seasonal trend of midday stem and leaf water potentials between stress and control treatments. The seasonal trend of leaf gas exchanges at noon was not affected in all years, however transpiration showed lower values ($p < 0.05$) at 3p.m. at 65 DAFB (2020) and 76 DAFB (2021). Stressed fruit presented a lower weight throughout all the season in 2020 while in 2021 this difference was observed only at harvest. Shoot growth was significantly lower in stressed plants in 2020 while in 2021 a similar growth was observed. Quality traits at harvest showed similar values. Flesh tissues from fruit subjected to water stress showed a higher accumulation ($p < 0.05$) in total phenolic content in 2020. The lack of water deeply modified the fruit metabolome landscape. In particular, stressed fruit showed a higher accumulation of lipids and carbohydrates compared to control samples. On the other hand, a repression of cinnamic and carboxylic acid, and terpenoid metabolites was observed in comparison to control fruit. These results lead to the conclusion that pre-harvest irrigation can be successfully applied on sour cherry, without penalizing plant physiological

performances and could be used as a tool to modulate the fruit metabolome, although the final fruit size was negatively affected.

INTRODUCTION

Among the allarming factor derived by climate change, one of the most threatening is related to the alterations in the water cycle (IPCC, 2014). Precipitation fluctuations are indeed becoming irregular and unpredictable, and extreme weather events such as droughts, floods, and tropical storms are more common (Hatfield et al., 2011). Besides precipitations, rising temperatures affect water availability and crop water requirements. Fruit tree crops are highly influenced by climate changes, since the stress to which plants are subjected modify their physiological performances (Zandalinas et al., 2022). These factors can potentially lead to species decline or even disappearance from certain areas due also to water scarcity (Anderegg et al., 2015). Sustainable water management can be an alternative to assure both food security and agricultural land preservation.

According to FAO, sour cherry global production in 2020 was 1'479'045 million tons. Russia (254,800 tons), Turkey (189,184 tons), Ukraine (174,630 tons), Serbia (165,738 tons), Poland (153,100 tons), and Iran (121.651 tons) are the top producers.

Sour cherry is charcterized by three stages of fruit development, as all the others stone fruit crops. Rapid cell division characterizes stage I, while during stage II fruit slow down their growth rates and pit hardening occurs. Stage III is characterized by cell expansion and fruit ripening (Papenfuss and Black, 2010).

The total water amount of sweet cherry tree can depend on several factors such as soil conditions, cultivar and rootstock genotypes, and the geographical area considered. In fact, high spring temperatures increase plant evapotranspiration and may lead to the need of water supply. Annual irrigation amount ranged from 600-630 mm in Lleida, Spain (Marsal et al., 2010) to 750-1000 mm in Washington State, USA (Hanson and Proebsting, 1996). Sour cherry require a similar amount of water during

the plant and fruit developmental and maturation cycle (Papenfuss and Black, 2010). When sour cherry is grown in loamy and clay soil water supply is generally unnecessary until late stage II (Papenfuss and Black, 2011).

Similarly to other fruit crop, sour cherry water needs are not constant during the season, since they are influenced by crop phenology and by the environmental conditions. In addition, it has been shown that fresh weight and yield of sour cherry fruit at harvest were not dramatically affected by the irrigation regimes, although a small increase in undersized fruits was found on plants subjected to deficit irrigation (Papenfuss and Black, 2011). However, soluble solids concentration and colour intensity increased with the severity of the irrigation deficit (Papenfuss and Black, 2011). Thanks to its high phenyloproanoid metabolites content, sour cherry fruit extracts are characterised by a high antioxidant activity (Ferretti et al., 2010). Nevertheless, few exaustive information are available regarding the specialized (secondary) metabolite lansdcapes in these species. Several phenolic compounds were detected in sour cherry cultivars, including flavan-3-ols and tannins, anthocyanins, hydroxycinnamic acids, flavonols and flavons metabolites (Wojdyło et al., 2014). The antioxidant activity of a growing fruit is subjected to many changes due to the variation on phenological phases and pedoclimatic environment. In general, significant increases of phenols and flavonoids, well known antioxidants (Behr et al., 2020; Nakabayashi et al., 2014), take place during ripening stage in fruit crops (Allegro et al., 2021; García-Gómez et al., 2022). Under stress condition, plants produce oxygen radicals and other reactive oxygen species (ROS) that contribute to the increase of oxidative stress in the plant (Mittler et al., 2022). ROS signalling enhcance the accumulation of several antioxidant metabolic, such as anthocyanins and other flavonoids (Naing and Kim, 2021). In fact, transgenic plants overaccumulating anthocyanin showed higher tolerance to water and salt stress compared to wild-type plants (Naing et al., 2017).

Sour cherry fruits are mainly consumed after industrial processing, because of their perishability and their low SSC/TA ratio (McCune et al., 2011). The potential positive effects of sour cherry dietary intake have been assessed . A review work by Ortega et al. (2021) analysed many studies evaluating the effect of sour cherry on muscle

damage and suggesting that it is potentially able to reduce the time of recovery of functional performance variables, perceptual variables and inflammation (Ortega et al., 2021). A link between sour cherry intake and a reduction of risks of inflammatory disease was also established (Kelley et al., 2018; Mansoori et al., 2021). Sour cherry extract could also reduce oxidative stress and markers of muscle and cardiac damage following intense resistance exercise (Hooper et al., 2021), reduce systolic blood pressure of hypertensive men (Keane et al., 2016) and had promising effects in attenuating the adipogenesis on rats (Cocci et al., 2021). It has also been found to positively affect sleep duration thanks to an increase of exogenous melatonin (Howatson et al., 2012).

From an agricultural point of view there is the increasing need to understand fruit tree physiology for a better plant adaptation and to secure fruit production. On the other hand, sour cherry fruit is characterized by nutraceutical compounds that can have positive implication on human health, but their composition is influenced by the genotype, the area of production and on the management practices applied. To date, there is a lack of information about the relationship between plant physiological performance and final fruit quality, also in terms of nutraceuticals, under drought conditions. In this work we charcaterized the effect of water deficit irrigation on the physiology and specialized metabolites (nutraceuticals) composition on sour cherry fruit. The plant physiological behaviour showed that sour cherry can afford water deficit during stage III of growth increasing its total phenolic content, thus coupling the need for climate change adaptation to potential health benefits and intrinsic quality of the fruit production.

MATERIALS AND METHODS

Plant material and experimental design

The trial was conducted during 2020 and 2021 vegetative seasons at the experimental farm of the University of Bologna, Italy (44°33’01.7’’N, 11°23’16.5’’ E). The climate of the area is sub-continental, with an average annual temperature of

15.6 °C and an average annual precipitation of 756.5 mm (Istat.it). The sour cherry cultivar used in the trial was "Rio Cerca", a self-fertile cultivar belonging to Amarelles group, one of the main categories in which these fruits are divided (Palonen et al., 1998). The orchard was planted in 2010 and resulted composed by 3 rows north-south oriented, with a spacing of 1.5 meters between single trees and 4.0 meters among rows, for a density of 1666 trees ha^{-1}. In both years, two irrigation treatments were imposed :100% and 0% of crop evapotranspiration rate. The trials were set up on 11th May in 2020 and on 28th April in 2021. In 2020, on parcel per treatment was set in the central row of the orchard while in 2021, two blocks per treatment were considered. Three plants per block were monitored during the entire period. Starting from 2nd June 2020 and 1st June 2021, irrigation was stopped in half of the blocks, while the rest of the plants were maintained at a standard irrigation level (100% of ETc) until fruit harvest. Crop water requirements were calculated thanks to the irrigation scheduling system provided by Emilia Romagna region (www.irriframe.it). Data were collected from a weather station positioned inside the orchard (Winet s.r.l., Cesena, Italy). Irrigation was applied through drip system, using micro sprinklers and arranging a by-pass structure to exclude the selected trees from the irrigation. The flow rate in control was kept constant at 1.7 mm h^{-1} and provided regularly. Orchard management, including fertilization and pest management, were done according to common commercial practices. Full bloom occurred on 10th and 7th April in 2020 and 2021, respectively, and fruits were harvested on 16th and 28th June (67 Days After Full Bloom [DAFB] in 2020 and 82 DAFB in 2021).

Physiological measurements

Water relations

Plant water status was assessed through the measurement of stem (Ψ_{stem}), leaf (Ψ_{leaf}) and fruit (Ψ_{fruit}) water potentials. These parameters were detected at 45 and 62 DAFB in 2020 and at 41 and 76 DAFB in 2021. Measurements were take at 9 a.m., 12 p.m. and 3 p.m.. A Scholander pressure chamber (Soilmoisture Equipment Corp., Goleta, Santa Barbara, CA, USA) was used to measure water potentials. To measure leaf water potential, five well exposed leaves were collected and immediately

analysed for each treatment as reported by Turner and Long's (Turner and Long, 1980). Five additional leaves were chosen from the inner part of the canopy, in the closest position to the trunk, to evaluate stem water potentials. In this case, leaves were firstly covered with an aluminum foil and enclosed in a plastic bag for at least 90 minutes before the measurement. After that, they were excised and water potentials were immediately measured, according to the methodology described by McCutchan and Shackel (McCutchan and Shackel, 1992) and by Naor et al. (Naor et al., 1995). Fruit water potential were similarly assessed using fruits pedicels. Because of high fruit softness, it was not possible to assess fruit water potentials at 72 DAFB 2021.

Leaf Gas Exchanges

Photosynthetic rate, stomatal conductance and leaf transpiration were measured using a Li-COR 6400 (LI-COR, Lincoln, NE, USA), an open-circuit infrared gas exchange analyzer fitted with a LED light source. A well-exposed leaf per tree was installed on Li-COR, setting air CO_2 concentration at 400 ppm and keeping light intensity constant on the basis of the natural irradianc). Six measurements per treatment were carried out on the same trees in concurrence with the water potential measurements.

Fruits and shoots growth

Fruits and shoots growth parameters were regularly monitored during the growing season. Four fruit and two shoots per tree were selected and tagged in 2020, while in 2021 the number was increased up to 8 fruit and 4 shoots per tree, for a total of 48 fruits and 24 shoots per treatment. They were in well-exposed positions and equally distributed on both sides of the canopy. Fruits measurements were made at 40, 45, 56, 62 and 67 DAFB in 2020 and at 29, 35, 41, 48, 55, 62, 69, 82 DAFB in 2021. Shoot growth was measured at the same dates. A digital caliper equipped with a data logger was used to measure fruit growth rate. Shoots growth was assessed with a measuring tape. Collected data were lately used to calculate Absolute Growth Rate (AGR) using the following equations:

$$AGR_{t1} = \frac{FW_{t1} - FW_{t0}}{t_1 - t_0}$$

Where: FW = fruit weight; t1 = recording time; t0 = previous recording time.

The fruit diameters measured were then converted into weight using the following equation:

$$W(g) = a \cdot D\ (mm)\text{^}b$$

Where W = fruit weight; D = diameter; a = 0.0009; b = 2.8342

The two constants "a" an "b" were obtained by regressing diameter and weight data of about 130 fruits picked from the same sour cherry orchard of this experiment in 2020 and 2021. The R^2 value of the relationship between the two parameters was > 0.97.

Statistical analysis

For the collected data averages and standar error were calculated. A t-test analysis between the growing conditions (control vs treatment) was performed. Statistical significance was considered when p value < 0.05.

Fruit quality determination

Fruit quality

Solid Soluble Content (SSC, °Brix) was measured on 30 sour cherries per treatment on 16th June 2020 (67 DAFB) and on 28th June 2021 (82 DAFB). Fruit were harvested at the same ripening stage in both years. Acidity (%) was measured at 82 DAFB in 2021 using the digital refractometer (Digital Hand-held PAL-1, ATAGO).

Total phenolic content (TPC)

Total phenolic content (TPC) was measure in fruits harvested at two dates in 2020 and in 2021. Twelve fruit per treatment were collected from 3 different plants. Then, samples were immediately brought to the laboratory, the skin and the pulp separated, immediately frozen in liquid nitrogen and stored at -80°C. The TPC was evaluated according to the Folin-Ciocalteau method proposed by Cantín et al. (Cantín et al., 2009), adapted from (Singleton et al., 1965). Briefly, 0.5 g for skin and 1 g for flesh of freezed material were homogenized with an homogenizer (IKA T252 ULTRA-TURRAX) for 2 minutes on ice, with 10 mL of extraction solution, consisting of 0.5N HCl in methanol/Milli-Qwater (80%v/v). The mixture was then incubated overnight at 4 °C and the following day centrifuged for 20 min at 4 °C and 20000g. Supernatant was recovered, and the volume measured. 500 µL of the extract was diluted in water with 500 µL of Folin-Ciocalteu's reagent. After 3min of reaction, 1mL of 1N sodium carbonate (Na_2CO_3) was added. The tubes were mixed for 15 s and then put in the dark for 60 min at 20 °C. Absorbance was measured at 725 nm using a spectrophotometer (Biochrom). The standard calibration curves were daily prepared using gallic acid (3,4,5-trihydroxy- benzoic acid). The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight.

Anthocyanins quantification via HPLC

A quantity of 25 ml of extracts (prepared according to the methodology used TPC determination) were first concentrated through distillation using the RotaVapor (Heidolph) and then re-suspended in 1 ml of extracting solution. 200 µL of the supernatant transferred to HPLC auto-sampler vials. Samples were analysed a Waters 1525 HPLC (Waters, Milford, MA, USA) equipped with a diode array detector (DAD) and a Phenomenex (Castel Maggiore, Bologna, Italy) reversed-phase column (RP18, 250 mm 4 mm, 5 µm). Anthocyanin identification was conducted comparing their elution order and matching the spectra of identified peaks with anthocyanin compounds retrieved from published papers (Sokół-Łętowska et al., 2020). Anthocyanin quantifications were based on spectra at wavelength of 520 nm and expressed as cyanidin-3-O-glucoside equivalents (Extrasynthese, Genay, France), as commonly reported (among others, Homoki et al., 2016).

Untargeted Metabolomic analysis

Specalized metabolite extraction and untargeted metabolomic analyses were performed according to Boutet et al. (2022) and Routaboul et al. (2006).

Metabolite extraction. Part of the frozen material was lyophilized for following metabolomic analysis. Specialized metabolites were extracted from 10 mg of lyophilized skin and flesh tissues. In brief, 1.5 mL of MeOH/Acetone/H2O/TriFluoroAcetic acid (30/42/28/0.1 v/v/v/v) and 500 ng of Apigenin (used as internal standard) were added to each sample which was then homogenized in 2-ml tubes using a FastPrep device (2 rounds x 0.30 sec, 6 m/sec). The mixtures were then shaken for 30 min at 4°C using a ThermoMixerTM C (Eppendorf), placed in an ice-cooled ultrasonication bath for 2 min, and centrifuged for 1 min at the maximum speed to remove debris. The surnatant was then placed in a new tube, and the extraction procedure repeated for a second time adding 1 mL of extraction solution to the tube containing the pellet after centrifugation. The phases obtained were dried down in a SpeedVac vacuum concentrator and resuspended in 200 µl of water and ACN 10% and filtered.

LC-MS/MS analysis. Untargeted metabolomic data were acquired using a UHPLC system (Ultimate 3000 Thermo) coupled to quadrupole time of flight mass spectrometer (Q-Tof Impact II Bruker Daltonics, Bremen, Germany). For untargeted polar and semipolar metabolomic analysis, a Nucleoshell RP 18 plus reversed-phase column (2 9 100 mm, 2.7 µm; Macherey-Nagel) was used for chromatographic separation. The mobile phases used for the chromatographic separation were (A) 0.1% formic acid in H2O and (B) 0.1% formic acid in acetonitrile. The flow rate was of 400 µL/min and the following gradient was used: 95% of A for 1-min, followed by a linear gradient from 95% A to 80% A from 1 to 3-min, then a linear gradient from 80% A to 75% A from 3 to 8-min, a linear gradient from 75% A to 40% A from 8 to 20-min. 0% of A was hold until 24-min, followed by a linear gradient from 0% A to 95% A from 24 to 27-min. Finally, the column was washed by 30% A at for 3.5min then re-equilibrated for 3.5-min (35-min total run time). Data-dependent acquisition (DDA) methods were used for mass spectrometer data in positive and negative

electro spray ionization (ESI) modes using the following parameters: capillary voltage, 4.5kV; nebulizer gas flow, 2.1 bar; dry gas flow, 6 L/min; drying gas in the heated electrospray source temperature, 140ºC. Samples were analysed at 8Hz with a mass range of 100 to 1500 m/z. Stepping acquisition parameters were created to improve the fragmentation profile with a collision RF from 200 to 700 Vpp, a transfer time from 20 to 70 µs and collision energy from 20 to 40 eV. Each cycle included a MS full scan and 5 MS/MS CID on the 5 main ions of the previous MS spectrum.

LC-MS/MS data processing. The data files (Bruker Daltonics, Bremen, Germany) obtained were first converted to .mzXML format using the MSConvert software (ProteoWizard package 3.0; Chambers et al., 2012). Data processing, mass detection, chromatogram building, deconvolution, isotope profiling, samples alignment and data export were performed using MZmine 2.53 software (http://mzmine.github.io/) for data files from both positive and negative ionisation mode. The ADAP chromatogram builder (Myers et al., 2017) method was used with a minimum group size of scan 4, a group intensity threshold of 6000, a minimum highest intensity of 6500 and m/z tolerance of 8 ppm. Deconvolution was performed with the ADAP wavelets algorithm using the following setting: S/N threshold 8, peak duration range = 0.01–2 min, RT wavelet range 0.01–0.8 min, MS2 scan were paired using a m/z tolerance range of 0.15 Da and RT tolerance of 0.1 min. Then, isotopic peak grouper algorithm was used with a m/z tolerance of 8 ppm and RT tolerance of 0.1. All the peaks were filtered using feature list row filter keeping only peaks with MS2 scan. The alignment of samples was performed using the join aligner with an m/z tolerance of 8 ppm, a weight for m/z and RT at 1, a retention time tolerance of 0.1 min. Metabolites intensity was normalized according to the quantity of internal standard (apigenin) and weight of fruit tissue (10 mg) used for the extraction.

Metabolite annotation and molecular network. A first research in the library with Mzmine was done, with identification module and 'custom database search' to begin the annotation with our library, currently containing 99 annotations (RT and m/z) in positive mode and 67 in negative mode, with RT tolerance of 0.3 min and m/z tolerance of 0.0025 Da or 6 ppm. This library research was repeated with the library containing references to the exact mass of 637 and 475 compounds in positive and

negative mode, respectively. In addition, it has been used the identification module and "Adduct search", adding to the list the mass of H_2O. Molecular networks were generated with MetGem software (Olivon et al., 2018a; https://metgem.github.io) using the .mgf and .csv files obtained with MZmine2 analyses. The molecular network was optimized for the Positive Electro Spray Ionization (ESI+) and Negative Electro Spray Ionization (ESI-) datasets, and different cosine similarity score thresholds were tested. ESI- and ESI+ molecular networks were generated using cosine score thresholds from 0.7 to 0.9, depending on the samples analyzed. Molecular networks were exported to Cytoscape software (Shannon et al., 2003; https://cytoscape.org/) to format the metabolic categories. Using the available MS2 spectral libraries (Massbank NA, GNPS Public Spectral Library, NIST14 Tandem, NIH Natural Product, and MS-Dial) the ESI- and ESI+ metabolomic data were compared for molecular network analyses with an absolute m/z tolerance of 0.02, four minimum matched peaks, and a minimal cosine score of 0.8. After that, chemical families were assigned to unannotated metabolites that are part of molecular network clusters that also contain annotated metabolites from steps 1 and 2. Finally, Sirius software (https://bio. informatik.uni-jena.de/software/sirius/) was used for metabolites that lacked or had unclear annotation. Sirius is based on machine learning techniques that suggest structures of unknown compounds using MS/MS data from chemical databanks and existing chemical structures. However, to some clusters a it was not possible to find a definite metabolomic category for all the clusters a s well as to isolated metabolites and those ones remained categorized as "not annotated".

Statistical and metabolic class -enrichment analyses. To assess statistical differences in metabolic feature intesities in fruit from Stress treatment against those from Control treatment, an ANOVA (p-value < 0.1) was performed using the Metaboanalyst software (Pang et al., 2022; Xia et al., 2009; MetaboAnalyst). The enrichment analyses to identified over-repressented metabolic categories were performed by using a hypergeometric test, and the p-value was controlled across all the treatments at level 0.05. The metabolite reference set was defined from the annotated and unknown metabolic categories. The analyses were performed with the software R.

RESULTS

Water relations

The daily trend of leaf and stem water potentials during the 2020 and 2021 seasons was monitored in fully and not -irrigated plants and fruits (Figure 1). In 2020, at 45 DAFB fruit from fully irrigated trees reached values of – 1.74 MPa compared to the -1.27 MPa of the not irrigated ones. At 62 DAFB, fruit from control trees maintained steady values around -1.65 MPa while the stressed ones showed lower values of -1.99 MPa. The 2021 season showed similar patterns between fully and not-irrigated plants and fruits (Figure 1b and 1d). At 41 DAFB plant water status was monitored before the stop of the irrigation, for this reason only data from the 100% treatments are shown (Figure 1b). At 76 DAFB all water potentials showed lower values than the previous monitoring period, with no differences between treatments.

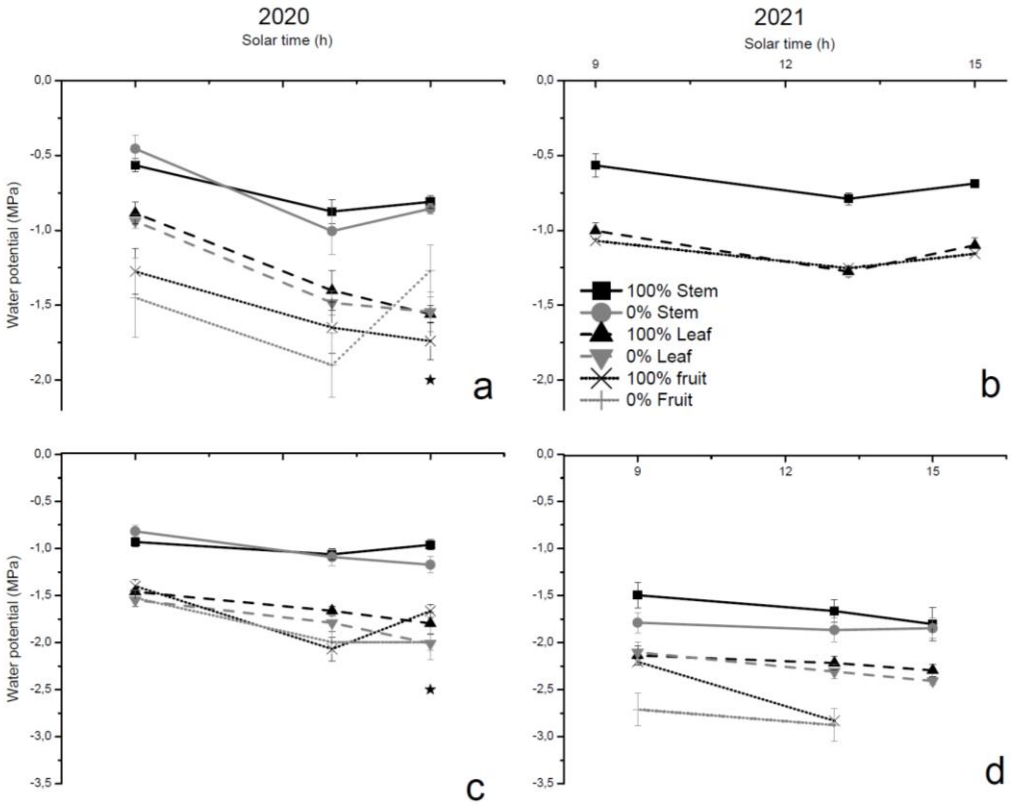

Figure 1. Stem (circles), leaf (triangles) and fruit (crosses) water potential at 45 (a) and 62 (c) DAFB in 2020 and at 41 (b) and 76 (d) DAFB in 2021, respectively, in 100% (black lines) and 0% (grey lines). Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

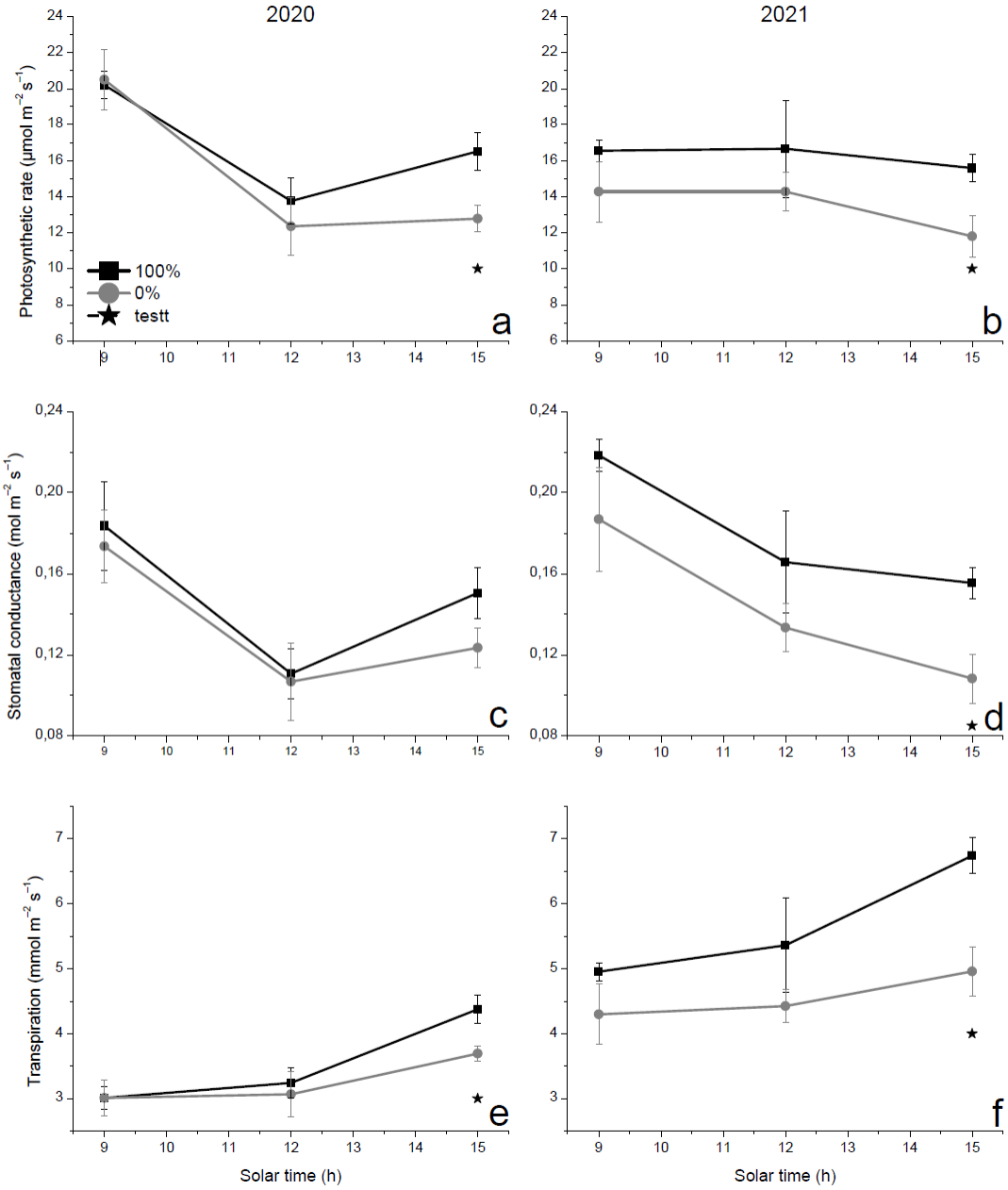

Figure 2. Photosynthetic rate (a, b), stomatal conductance (c, d) and transpiration (e, f) in 100% (black lines) and 0% (grey lines) in 2020 (62 DAFB) and 2021 (76 DAFB). Stars represent statistically significant differences at $p<0.05$ resulted by t-test.

Leaf Gas Exchanges

The daily patterns of leaf gas exchanges were monitored at different fruit developmental stages during both seasons. In the first monitoring date, at 45 DAFB in 2020 and at 41 DAFB in 2021, no significant differences were measured for all the monitored parameters (data not shown). The photosynthetic rate showed differences at 62 DAFB in 2020 and 76 DAFB in 2021, with values of 16.5 μmol m^{-2} s^{-1} and 12.7 μmol m^{-2} s^{-1} in 100% and 0% treatment, in 2020 and 15.5 μmol m^{-2} s^{-1} (100%) and 11.8 μmol m^{-2} s^{-1} (0%) in 2021 (Figures 2a and 2b). While stomatal conductance showed similar values between treatments in 2020, in 2021 0% treatment showed values of 0.11 mol m^{-2} s^{-1} while the control was 0.16 mol m^{-2} s^{-1} (Figures 2c and 2d). Leaf transpiration showed a progressive increase during the day with differences between treatments at 3 p.m. for both years. In fact, in 2020 0% treatment showed a lower traspiration with values of 3.7 mmol m^{-2} s^{-1} against 4.4 mmol m^{-2} s^{-1} of control trees. In 2021, although the absolute values were higher compared to the previous year, the trend was similar, with the 0% and the full irrigated trees showing values of 5 mmol m^{-2} s^{-1} and 6.7 mmol m^{-2} s^{-1}, respectively (Figures 2e and 2f).

Seasonal shoot and fruit growth

Shoot growth patterns differed between the two years. In 2020, shoot elongation was lower in drought-stressed compared to control samples at all monitored dates (Figure 3a). At harvest, despite the differences in the absolute values among fully irrigated and drought-stress samples, shoots growth rates were comparable between the treatments (Figure 3c). During the season 2021, the two irrigation treatments shared a similar shoot elongation and growth rates (Figures 3b and 3d).

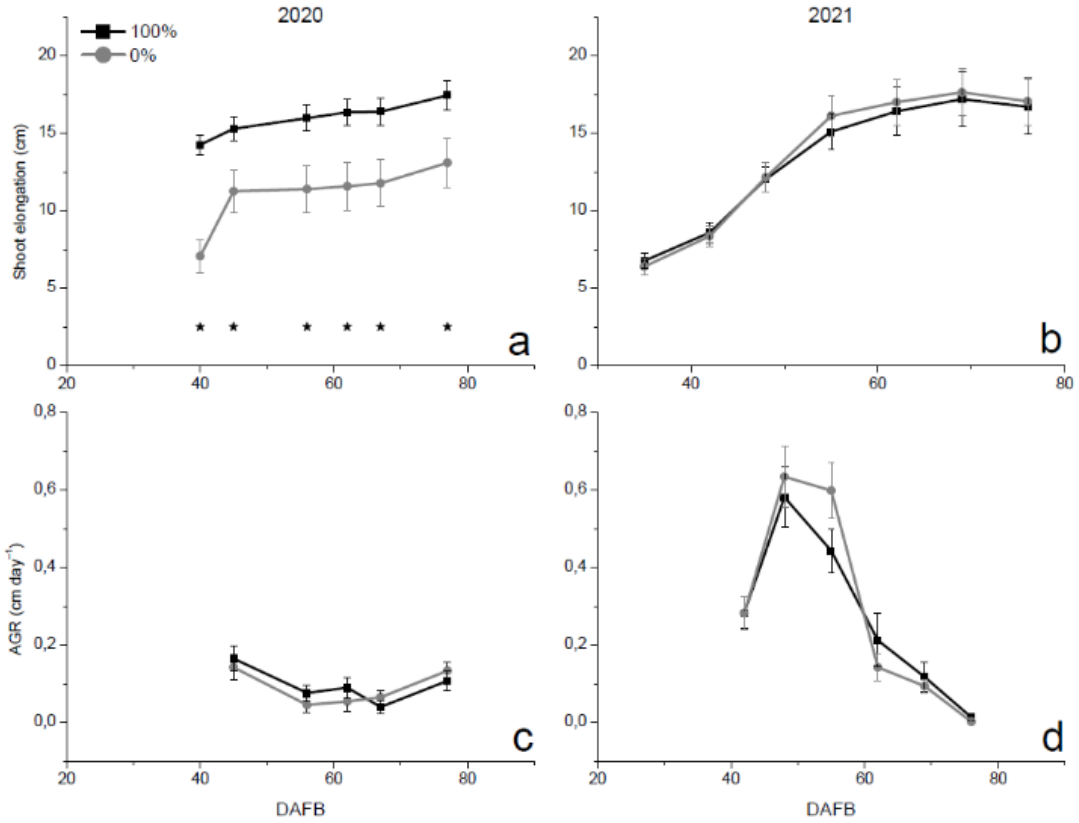

Figure 3. Seasonal shoot growth (a, b) in 100% (black lines) and 0% (grey lines) and AGR (c, d) in 2020 and 2021. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

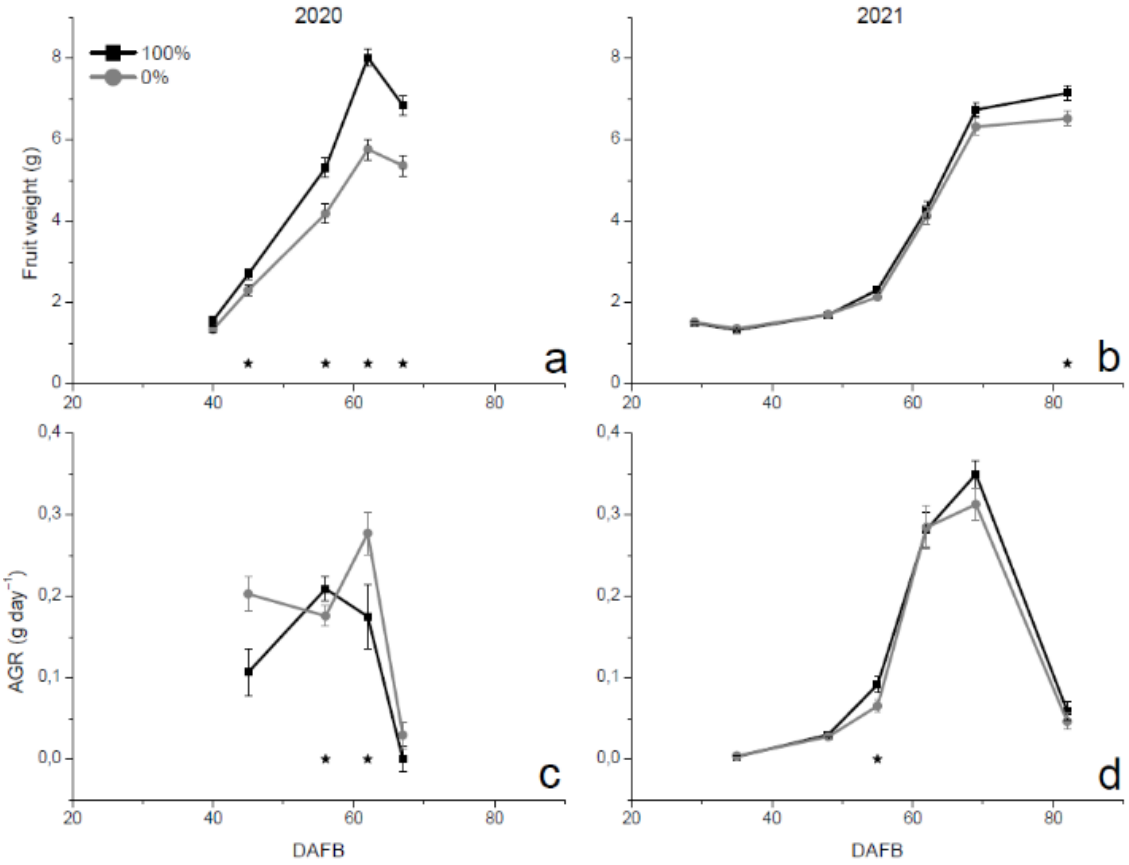

Figure 4. Seasonal fruit growth (a, b) in 100% (black lines) and 0% (grey lines) and AGR (c, d) in 2020 and 2021. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

Sour cherry fruit growth (Figure 4) presented different patterns depending on the year considered. In 2020 weight of drought-stressed fruits presented statistically significant difference from fully irrigated ones from 45 DAFB onwards (Figure 4a). At harvest, fruit weight was 5.4 g and 6.8 g for 0% and 100% irrigation, respectively. The AGR showed two moments with statistically significant differences: at 45 DAFB 2020 the growth rates were of 0.18 g day^{-1} and 0.21 g day^{-1} in 0% and 100% respectively, while at 62 DAFB the higher rates of growth were shown by the 0% treatment with values of 0.28 g day^{-1} while the control presented the same value of 0.18 g day^{-1}

(Figure 4c). In 2021 a similar weight and growth rate was observed between fully irrigated and drought-stressed fruits (Figures 4b, 4c).

Fruit quality at harvest

Table 1. Sour cherry fruit quality at harvest in 2020 and 2021. Fruit caliber, weight, soluble solids content (SSC, °brix) and acidity (only in 2021) were measured. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

	2020		
	Caliber (mm)	**Weight (g)**	**SSC (°Birx)**
100%	23.2	6.8	19.2
0%	21.3	5.4	19.7
Sign.	*	*	n/s

	2021		
	Caliber (mm)	**Weight (g)**	**SSC (°Birx)**
100%	23.7	7.1	19.8
0%	22.9	6.5	19.8
Sign.	*	*	n/s

At harvest, fruits from plants subjected to waer deficit showed lower diameter and weight compared to those harvested from fully irrigated plants in both 2021 and 2021 (Table 1). Soluble solid content seemed to be unaffected by the irrigation regime applied (Table 1).

Fruit total polyphenol and anthocyainin quantification

Total polyphenols (TPC) and anthocyanins contents were measured in skin and flesh. In 2020, TPC was higher in non-irrigated fruits at harvest (75 DAFB) compared to fully-irrigated fruits (Tab.2). No differences were observed for fruit harvested in 2021. At the first sampling date (72 DAFB) of 2021 the absolute values were higher with respect to the previous growing season but at harvest (82 DAFB) the values measured were dramatically decreased in both treatments.

Table 2. Total phenolic content in sour cherry flesh and skin tissues, in 2020 and 2021. Values are expressed as mg of GAE g^{-1} of frozen tissue. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

Flesh	2020				2021			
	60 DAFB	SE	**75 DAFB**	SE	**72 DAFB**	SE	**82 DAFB**	SE
100%	**175.9**	40.7	**192.1**	34.5	**480.6**	58.7	**92**	13.3
0%	**309.1**	47.6	**319.2**	10.1	**491.8**	37	**80.5**	15
Sign.			*					

The anthocyanins measured in sour cherry belonged all to the group of cyanidin. In details, we found cyanidin-3-sophoroside (Cya-3-S), cyanidin-3-glucosilrutinoside (Cya-3-GR), cyanidin-3-glucoside (Cya-3-Gl) and cyanidin-3-rutinoside (Cya-3-R). Other unidentified peaks were observed, probably belonging to small anthocyanidin fractions. The signals corresponding to the four metabolites measured were summed up to calculate the total anthocyanidin content. In 2020, fruit skin showed statistically significant different values in Cya-3-S and Cya-3-Gl at 60 DAFB, with the highest content in the fruit from plants that received no-irrigation in the last part of the cycle (Tab.3). In the second sampling date, at 75 DAFB 2020, fruit skin of 100% treatment presented higher values in Cya-3-GR and Cya-3-R. At 75 DAFB 2020, fruit flesh showed values of 21.5 and 28.1 µg/g of frozen fruit in Cya-3-GR and of 2.4 and 4.3 µg/g frozen fruit in Cya-3-R , for 100% and 0% irrigated fruit, respectively. Total skin anthocyanin content was higher in control treatment at 82 DAFB 2021. Looking at the single anthocyanin fractions, Cya-3-Gl was higher in the 0% treatment (69.5 and 33.8 µg/g frozen fruit in 0% and 100%, respectively) while Cya-3-R presented higher values in 100% treatment (227.1 and 317.8 µg/g frozen fruit in 0% and 100%, respectively).

Table 3. Anthocyanin content in flesh and skin tissues of sour cherry fruit in 2020 and 2021. Cya-3-S: cyanidin-3-sophoroside; Cya-3-GR: cyanidin-3-glucosilrutinoside; Cya-3-Gl: cyanidin-3-glucoside; Cya-3-R: cyanidin-3-rutinoside. Stars represent statistical significant differences at $p<0.05$ resulted by t-test.

				Anthocyanin content (µg/ g frozen fruit)				
Year	Sampling date	Fruit tissue	Treatment	Cya-3-S	Cya-3-GR	Cya-3-Gl	Cya-3-R	Total anthocyanin content
2020	60 DAFB	Skin	100%	69.3	296.4	78.1	225.1	753.5
	60 DAFB	Skin	0%	157.3	294.8	199.6	297.9	1048.6
	Sign.			*		*		
	75 DAFB	Skin	100%	206.9	644.5	219.7	525.3	1830.1
	75 DAFB	Skin	0%	204.4	152.8	362.7	227.5	1202.7
	Sign.				*		*	
	60 DAFB	Flesh	100%	3.8	31.1	1.6	3.9	40.5
	60 DAFB	Flesh	0%	3.2	27.1	0.2	4.0	34.5
	Sign.							
	75 DAFB	Flesh	100%	1.9	21.5	0.2	2.4	26.0
	75 DAFB	Flesh	0%	4.5	28.1	0.5	4.3	37.4
	Sign.				*		*	
2021	72 DAFB	Skin	100%	67.2	293.9	68.0	233.7	756.6
	72 DAFB	Skin	0%	64.0	294.6	69.4	227.1	735.6
	Sign.							
	82 DAFB	Skin	100%	38.6	448.0	33.8	317.8	954.7
	82 DAFB	Skin	0%	64.1	294.5	69.5	227.1	735.6
	Sign.					*	*	*
	72 DAFB	Flesh	100%	2.3	5.9	3.3	2.3	15.9
	72 DAFB	Flesh	0%	3.3	9.0	1.7	2.9	16.8
	Sign.							
	82 DAFB	Flesh	100%	2.3	6.0	3.3	2.3	15.9
	82 DAFB	Flesh	0%	3.3	9.0	1.7	2.9	16.8
	Sign.							

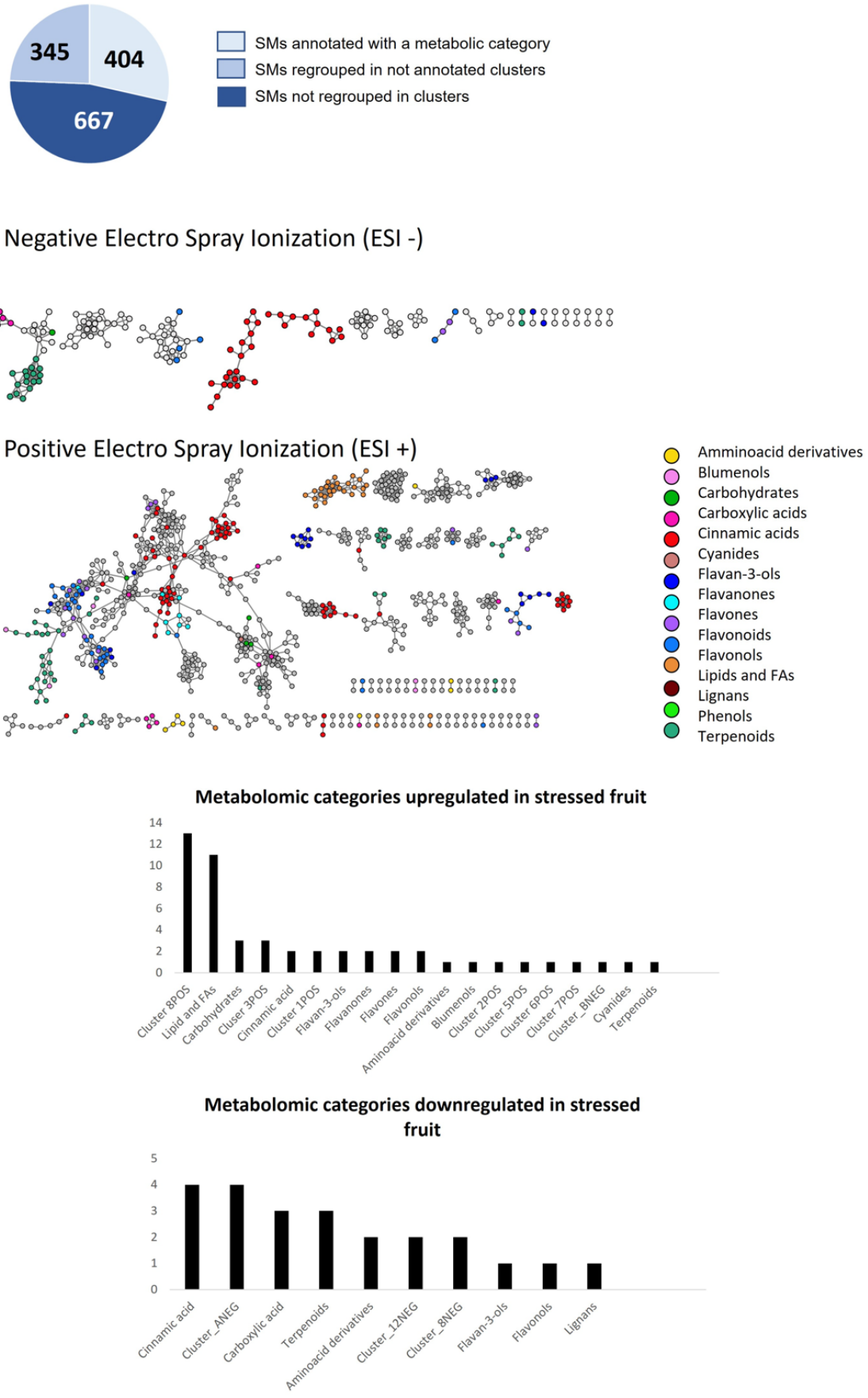

Figure 5. Molecular networks and annotation of the skin-specialized metabolites (SMs). (a) Numbers of SMs present in a molecular network with or without an annotation. (b) The molecular networks are shown for metabolite analyses carried out in positive (ESI+) and negative (ESI-) ionization modes. Different colours correspond to different metabolic classes. Metabolites are grouped based on their chemical structures. Cosine similarity scores of 0.8 and 0.75 were used for ESI+ and ESI-, respectively. (c) Histograms showing the upregulated and downregulated metabolic categories identified in sour cherry skin.

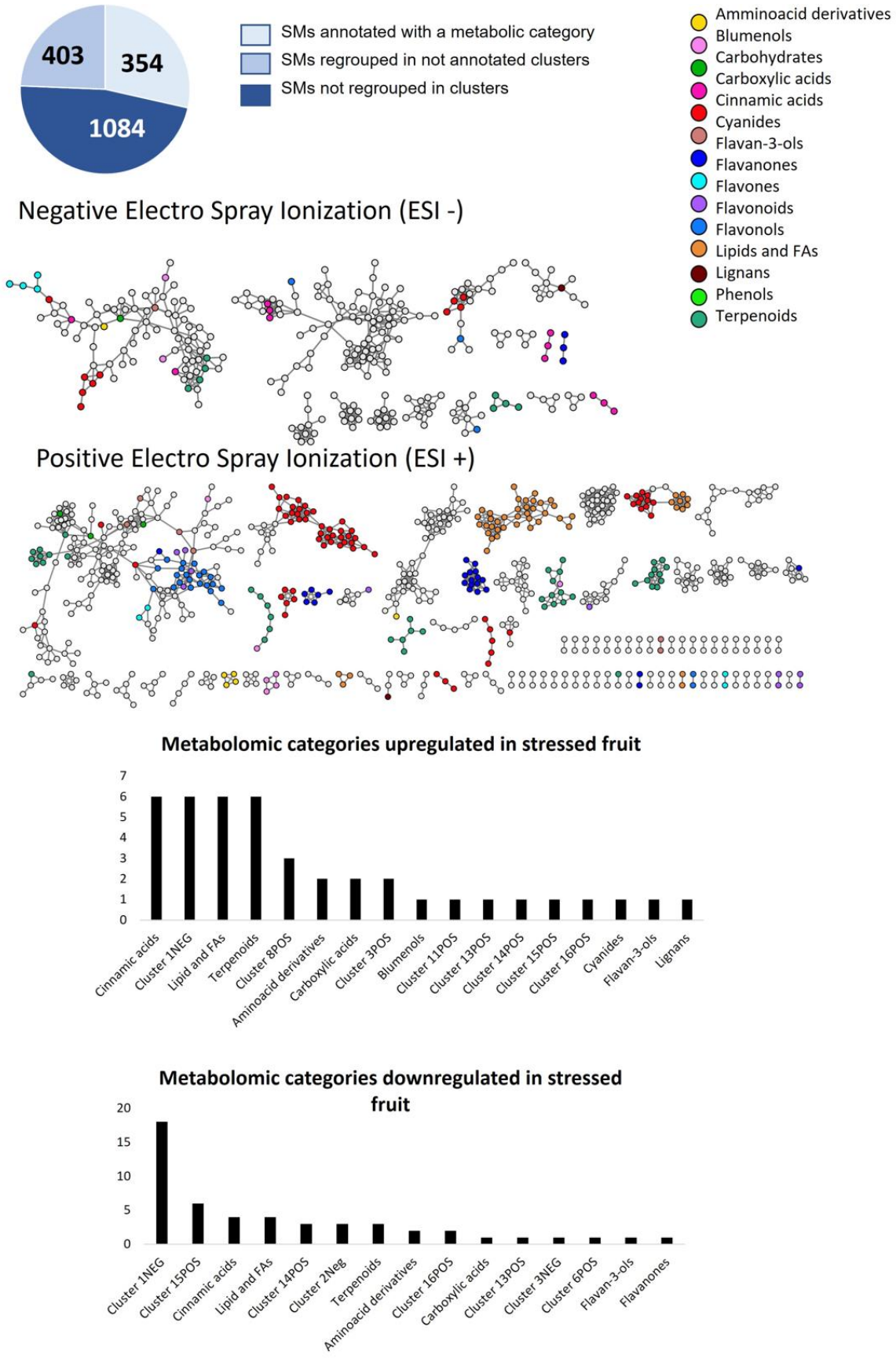

Figure 6. Molecular networks and annotation of the flesh-specialized metabolites (SMs). (a) Numbers of SMs present in a molecular network with or without an annotation. (b) The molecular networks are shown for metabolite analyses carried out in positive (ESI+) and negative (ESI-) ionization modes. Different colours correspond to different metabolic classes. Metabolites are grouped based on their chemical structures. Cosine similarity scores of 0.8 and 0.75 were used for ESI+ and ESI-, respectively. (c) Histograms showing the upregulated and downregulated metabolic categories identified in sour cherry flesh.

Specialized metabolite landscapes of sour-cherry fruits

Untargeted metabolomics (LC–MS/MS) was used to characterize the specialized metabolite (SM) diversity of sour cherry fruits collected at harvest 2021 (Figure 6 and 7). Metabolomic data from ESI+ and ESI- modes allwoed the detection of 1416 and 1849 peaks of putative known and unknown ions or metabolic features in the fruit skin and flesh, respectively. To improve the annotation of the detected metabolic features, the LC-MS/MS data were used to construct a molecular network (Figures 6 and 7). This method allows the clustering of compounds that have a high degree of structural similarity based on their MS2 spectra, and as a result, it enables the identification of clusters of unknown metabolites by making use of the MS/MS information for annotated compounds. (Olivon et al., 2018b) (see M&M for further details about the annotation procedure). The annotation of sour cherry metabolic features allowed the assignment of 29% and 22% of the detected molecules to a known metabolic category in skin and flesh, respectively. On the other hand, 24% (in the skin) and 19% (in the flesh) in of the metabolites were found to be included in clusters containing metabolic features that were not annotated. Finally, 47% and 59% of the metabolites were not assinged to a cluster and were not annotated in skin and flesh, respectively (Fig 6 and 7). Overall, SMs identified in sour cherry fruit have been assigned to 13 metabolic categories: flavonols, flavan-3-ols, flavones, flavanones, amino acids and derivatives, cinnamic acids, carbohydrates, carboxylic acids, blumenols, cyanides, terpenes, nucleosides and derivatives, and lignans. Some differences in the SMs intensity were observed in function of the irrigation treatment applied in the field. Enrichment analyses showed that the signal of carbohydrates and cinnamic acids, and of unidentified clusters 8POS, 3POS and 1POS was more intense in the skin of non-irrigated fruits compared to the fully irrigated ones. Some metabolic features were also found to be downregulated in fruit from 0% plants when compared to 100% fruit: cinnamic acids, carboxylic acids and terpenoids were the categories more represented. In sour cherry flesh the categories found to be upregulated in 0% fruit were cinnamic acids and terpenoids as well as cluster 1NEG. The molecules downregulated instead belonged to cluster 1NEG, 15 POS, cinnamic acids and lipids and fatty acids.

DISCUSSION

Total precipitations from the beginning of the trial to harvest corresponded to 68 mm in 2020 and to 18.3 mm in 2021. However, the lack of irrigation led a to a significant decrease exlusively in the Ψ_{fruit} at 3 p.m. in 2020 (Figure 1). This might suggest how fruit represents a strong "sink organ" during the growing season, since their capability to maintain a low water potential made easier for them to attract assimilates. However, in 2021 the irrigation level did not induce any differences in the water potential of the different tree organs, although the absence of irrigation was even longer than the previous year. On the contrary, in both years, leaf gas exchanges, were negatively affected by deficit irrigation , especially in the late afternoon (at 3 p.m) except for stomatal conductance in 2020, which kept values similar to those of 100% plants. Thus, trees were able to sustain high leaf gas exchanges in the morning and at midday even though soil water availability was reduced, while carbon assimilation was decreased in the afternoon. Suprisingly, leaf gas exchanges seems to be modulated by to soil water status rather than stem water potential which is usually considered the best indicator for plant water status (Shackel, 2007).

Regarding vegetative growth, shoot presented similar growth patterns between the years (Figure 3). However, in 2020 shoot length in deficit irrigated trees was already lower than controls from the first monitoring date. This must be due to a sampling error and/or to an undersized sampling number. However, no difference were recorded in the shoot absolute growth rate between the two treatments. During 2021, no differences were recorded between treatments. This was probably due to the larger sample set, that decreased their variability. During the season shoot growth pattern followed a quite linear trend that slowed down only approaching to harvest.

Seasonal fruit growth followed the double sigmoid curve, typical of stone fruits (Figure 4). In both years, 100% irrigated fruit showed higher diameter and weight compared to the 0% ones. Usually, fruit size is one of the main parameters responding to the lack of water. Similar results were found on sour cherry (Papenfuss and Black, 2011) and on sweet cherry (Blanco et al., 2019; Carrasco-Benavides et al. 2020), and on many other fruit crops (Venturi et al., 2021; Morandi et al., 2014; Peña et al., 2013; Gariglio et al., 2007; Naor et al., 2004; Torrecillas et al., 2000; Ebel et

al.,1993). However, yield was not affected since plants produced 8.5 ± 2.5 kg and 7.8 ± 2.7 kg in Control and Stress, respectively (data not shown). Soluble solids content (SSC, Brix°) at harvest revealed how water stress had no effects (Table 1). This result was not in accordance with (Marsal et al., 2010), which showed a negative effect of water stress on soluble solid concentration of sweet cherry. Total phenolic content showed contrasting results (Table 2). In fact, in 2020 an increase between the two sampling dates was observed, but in 2021 a drastic reduction between the first (72 DAFB) and the second (82 DAFB) sampling date was seen. Total anthocyanin content was not affected by the treatment in the majority of the cases. The values found in this study can not be easily compared to the ones found in the literature, since normally whole fruit are used for the analysis (Damar and Ekşi, 2012; Milošević et al., 2020; Mulabagal et al., 2009; Proietti et al., 2019; Sokół-Łętowska et al., 2020). This suggested that the values showed in the abovementioned works represented an average values of skin and flesh but our research point out that skin is richer in anthocyanins than the flesh. Our study underlined major differences in the anthocyanin content between skin and flesh tissues (Table 3). As a matter of fact, skin presented values from 18 to 70 times higher than the flesh, depending on the sampling date considered. Total anthocyanin content was higher in sour cherry skin at 82 DAFB 2021 for fruit from 100% plants. A similar trend was found also at harvest in 2020 although differences were not statistically significant. This phenomenon can be explained in light of the increase of daily temperatures which have been already found to negatively affect anthocyanin synthesis in apple (Lin-Wang et al., 2011), grape (de Rosas et al., 2017; Mori et al., 2005; Movahed et al., 2016), strawberry (Matsushita et al., 2016) and plums (Niu et al., 2017). The chromatograms revealed the presence of 6 anthocyanin fractions. The first four fractions were identified as cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside, cyanidin-3-sophoroside, and cyanidin-3-glucoside, as already confirmed by others previous works (Proietti et al., 2019; Sokół-Łętowska et al., 2020). According to literature, these two unclassified peaks could be peonidin-3-rutinoside and peonidin-3-rutinosidel gucoside (Blando, Scardino, De Bellis, Nicoletti, & Giovinazzo, 2005; Chandra et al., 2001; Kim et al., 2005), since we created a calibration curve with cyanidin-3-galactoside standard but no matches were found in the samples. The

irrigation treatment applied in the field influenced fruit anthocyanin composition in 2020. In fact, at 62 DAFB 2020 non-irrigated fruit presented higher values in the skin of cyanidin-3-sophoroside and cyanidin-3-glucoside, while at harvest cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside presented higher values both in the skin and in the flesh, without however a strong influence in the total amount. We discovered a large diversity of SM classes (16 in total) in sour cherry fruit thanks to the molecular network approach (Elie et al., 2019; Olivon et al., 2018) combined with manual confirmation of metabolite annotation using molecular structure identification (Duhrkop et al., 2019) (Figure 6 and 7). The current findings revealed that only 24% (in the skin) and 22% (in the flesh) of the identified metabolites are annotated or belong to a cluster with annotated metabolites. The metabolites present in public databases used for annotation account for a small portion of the plant metabolome (16% - 18%). It has to be noted that no antocyanin were identified with LC/MS-MS, probably because the aglycones weight was similar to those of other flavonoids (Abrankó and Szilvássy, 2014). The lack of irrigation has induced some modifications in the quantity of some metabolites, with 247 and 226 up- and down-regulated features in skin and flesh, respectively.

CONCLUSIONS

The results of this experimental trial showed that despite the lower fruit diameter and weight at harvest, sour cherry plants managed to resist to water deficit conditions without showing particularly intense deficiency and without compromising their physiological functions. Moreover, fruit sugar and acidity content were not affected by the irrigation treatment applied while total phenolic content was increased in 0% treatment. Water strees deeply modified fruit metabolomic landscape with an increase in lipids and carbohydrates as well as a drecrease in cinnamic and carboxylic acid and terpenoid metabolites. Although more studies are needed to confirm these results, the conclusion of our study is that the lack of irrigation in the last weeks before haverst can be successfully applied on sour cherry, without penalizing plant performances and could be also used as a tool to modulate the fruit metabolome. This investigation represents the first attempt to establish a

connection between plant physiological performance and fruit metabolomic composition in the context of water scarcity. Although more research is needed, this new information could be the basis for a novel strategy for sustainable and resilient fruit production that places less emphasis on fruit size and more on fruit nutraceutical traits.

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CHAPTER IV

APRICOT PLANT PHYSIOLOGY, FRUIT VASCULAR FLOWS AND FRUIT QUALITY UNDER PROGRESSIVE WATER STRESS.

ABSTRACT

Apricot is one of the most widely grown fruit crops in the world. Several studies have investigated the effects of deficit irrigation on apricot physiology as well as the fruit vascular flows characterization under no-limiting conditions. However, no information about the effect on plant physiology coupled with fruit vascular flows and fruit metabolome are available. This study assessed the effects of the lack of irrigation in the last weeks before harvest. Two irrigation treatments were imposed: "Stress", which completely stopped receiving irrigation during the last weeks before harvest, and "Control" that continued to receive 100% of ETc. During the growing season the following parameters were monitored: i) plant physiological parameters; ii) fruit vascular flows; iii) fruit quality; and iv) fruit metabolome on apricot cv. Farbela. Trees were able to afford long period in absence of irrigation without showing significant decrease in plant physiological performance as well as fruit growth over two years of study. This might be due to a possible "stress memory" but for sure the use of vigorous rootstocks characterized with deep root system enhanced the plant resilience to water stress. In addition, deficit irrigation seems to improve fruit quality in terms of sugar content, but the results shown in this study unfortunately do not provide strong demonstration of this relation. In conclusion, the irrigation scheduling in apricot is surely overestimated in the actual decision support system available in Emilia-Romagna region. Deficit irrigation protocols should be developed to optimize water use, fruit production and quality, especially in terms of nutraceutical components.

INTRODUCTION

Apricot is one of the most widely grown fruit crops in the world. According to FAO (www.fao.org), Turkey, Uzbekistan, Iran, Algeria, and Italy were the top producing countries in 2020. From an ecological point of view, apricot requires between 500 mm (Arzani et al., 2000) and 700 mm (Pérez-Pastor et al., 2007) of water to complete its vegetative and productive cycles. However, in the Mediterranean region the highest precipitation amount falls during the autumn and the winter periods, meaning that during summer there could be the need to restore plants from high evapotranspiration rate via irrigation. In order to maintain the actual productive performance in a sustainable way, a more precise irrigation scheduling can be a solution for both water savings and maintaining the crop productivity. Several studies on the application of regulated deficit irrigation (RDI) strategies have been done on fruit crops (Behboudian et al., 2011) and on apricot. These studies showed how the critical periods in which apricot productivity could be negatively affected by drought are the rapid fruit growth phase (stage III) and the early post-harvest period (Pérez-Pastor et al., 2009, 2007; Torrecillas et al., 2018, 2000). These findings are reported in several studies on the application of RDI strategies on apricot. Stress avoidance mechanisms such as stomatal control, epinasty, and limitation of transpiration by reducing leaf area are the primary contributors to apricot drought tolerance (Ruiz-Sánchez et al., 2000; Torrecillas et al., 1999). There is also a degree of osmotic adjustment in young apricot trees, but this adjustment is not observed in mature trees (Ruiz-Sánchez et al., 2007). Apricot trees usually respond to water shortages with an earlier stomatal closure (Henson et al., 1982; Torrecillas et al., 1988). On this regard, apricot trees are a good example of "embolism avoidance"(Nicolas et al., 2005), a response that is commonly interpreted as the result of efficient stomatal control (Cochard et al., 1996), typical of isohydric species.

Apricot fruit vascular flows have been studied by Giovannini et al. (2022). In their study it was reported how the xylem relative contribution to fruit growth was around 90% at the start of the season until 81 DAFB, and then decreased during fruit cell expansion and ripening. During the late season, phloem contributed 36% and 66% of daily fruit growth at 111 and 129. DAFB, respectively. In stage I, apricot had higher specific transpiration and xylem flow than peaches, but since phloem inflow was

lower, net fruit growth was half of that found in peaches (Morandi et al., 2007a). The same study reported that sap flow is directed towards the leaves in the morning and at midday, while in the afternoon the fruit were the ones receiving the highest inflows (Giovannini et al., 2022). Another parameter normally affected by water shortage is sap flow. It was found that young apricot trees had a significant sap flow reduction after 2 days of water withholding (Nicolas et al., 2005). Although some authors pointed out that the application of RDI strategies, if not managed correctly, can lead to lower fruit size and lower yield (Pérez-Pastor et al., 2014; Torrecillas et al., 2000), the majority of the studies that have been conducted up to this point show that apricot successfully responds to water stress (Pérez-Pastor et al., 2014). Nevertheless, it was found that color in fruit from plants subjected to RDI was more intense, leading to the hypothesis of an advancement of maturity due to the irrigation treatment applied (Pérez-Pastor et al., 2007). The application of RDI protocols can also result in an increase in fruit quality characteristics such as the total soluble solids and firmness (Pérez-Pastor et al., 2007; Pérez-Sarmiento et al., 2016). Apricot fruit are also rich in polyphenols (Fratianni et al., 2018). Several studies have assessed polyphenol content and antioxidant capacity on apricots (Erdogan-Orhan and Kartal, 2011; Fan et al., 2018), nectarines (Rodríguez-Calcerrada et al., 2015) and plums (Gil et al., 2002; Kim et al., 2003). Polyphenols eliminate free radicals, protect and regenerate other dietary antioxidants (e.g. vitamin E), and chelate pro-oxidant metals (Lima et al., 2014). Antimutagenic activity, cardiovascular disease risk reduction, and atherosclerosis protection are among their health benefits (Yao et al., 2004). In addition to their antioxidant properties, polyphenols in fruits are increasingly recognized as a crucial aspect of fruit quality because they have an impact on the color, flavor, and taste of the fruits (Crisosto, 2003).

To date, several studies on the effects of deficit irrigation on apricot physiology have been performed as well as the fruit vascular flows characterization under no-limiting conditions. However, no information about the effect of deficit irrigation on plant physiology coupled with fruit vascular flows and fruit metabolome are available. The objective of this study is to assess the effects of the lack of irrigation in the last weeks before harvest on : i) plant physiological parameters; ii) fruit vascular flows; iii) fruit quality; and iv) fruit metabolome on apricot cv. Farbela.

MATERIALS AND METHODS

Plant material and experimental set up

The study was conducted during the 2021 and 2022 vegetative growing seasons at the experimental farm of the University of Bologna, located in Cadriano, Bologna, Italy (44°32'53"N 11°24'45"E). The trial was set on a four years-old apricot orchard (Prunus armeniaca L.). The orchard was covered with anti-hail net. Trees were grafted on Myrobalan 29C (Prunus cerasifera Ehrh.) and trained at open vase, with a density of 1000 trees ha^{-1} (5 m x 2 m). Standard cultural practices were applied for pruning and fertilization; thinning was not performed in 2021 since spring frost reduced the initial crop load of about 50%. Irrigation was scheduled on the basis of the suggestions provided by the regional software platform "Irriframe" (Consorzio per il Canale Emiliano Romagnolo - CER). During the trial, two irrigation treatment were applied: "Stress", which completely stopped receiving irrigation, and "Control" that continued to receive 100% of ETc. In the "Stress" treatment the irrigation was closed on the 28th of June 2021 (108 DAFB) and on 3rd of June 2022 (83 DAFB) and remained closed until harvest, at 131 DAFB and 130 DAFB in 2021 and 2022, respectively. A total of 12 plants were considered in this study, 6 subjected to stress and 6 control, randomly divided in two blocks per treatment. Full bloom occurred on 12th March in both years and fruit were harvested on 21st July and 20th July in 2021 and 2022, respectively.

Measurements

Water potentials

Midday stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials were measured at 109, 112, 115, 117, 119, 122, 124, 129 and 131 DAFB in 2021 and at 88, 114, 124 and 129 DAFB in 2022, on at least 4 trees for each treatment, using a Scholander (Soilmosture Equipment Corp. Santa Barbara, U.S.A.) pressure chamber. Following Turner and Long's protocol (Turner and Long, 1980), one well-exposed leaf per tree (a total of six leaves per treatment) was selected and analyzed immediately after excision. To

measure stem water potential a leaf in the inner part of the canopy, as close to the trunk as possible, was chosen. In accordance with the methodology described by McCutchan and Shackel (1992) and Naor et al. (1995), the chosen leaf was then covered with aluminum foil and placed inside a plastic bag for at least 90 minutes to reach equilibrium.

Leaf gas exchanges

Leaf gas exchanges were measured in concurrence with the water potential measurements, using an open circuit leaf gas exchange system equipped with a LED light source (Li-COR 6400, LI-COR, Lincoln, Nebraska, USA). During each measurement, light intensity was maintained constant at the natural photosynthetically active radiation (PAR) level experienced by the leaves immediately before the measurements, and the CO_2 concentration was set at 400 ppm.

Fruit and shoot growth

Fruit and shoots were regularly monitored during the growing season 2022. On each tree, 8 fruit and 4 shoots were selected and tagged for a total of 48 fruit and 24 shoot per treatment. They were all in well-exposed positions, equally distributed on both sides of the canopy. Fruits measurements were made at 62, 73, 90, 103, 114, 124 and 129 DAFB. On the same days also shoot growth was measured. A digital caliper equipped with a data logger was used to measure fruit growth rate. Shoots growth was assessed with a measuring tape. Collected data were lately used to calculate Absolute Growth Rate (AGR) using the following equation:

$$AGR_{t1} = \frac{FW_{t1} - FW_{t0}}{t_1 - t_0}$$

Where: *FW = fruit weight; t1 = recording time; t0 = previous recording time.*

The fruit diameters measured were then converted into weight using the following equation from the literature (Giovannini et al., 2022):

$$W = a \cdot D \hat{} b$$

Where *FW = fruit weight; D = diameter; a = 0.0006; b = 2.9946* . The two constants “a” an “b” were obtained by the literature (Giovannini et al., 2022).

Fruit vascular flows

Fruit vascular and transpiration flows were determined at 115, 122 and 128 DAFB in 2021 and at 115, 125 and 130 DAFB in 2022, according to the methodology proposed by Morandi et al. (2014, 2010, 2007a, 2007b). In brief, fruit diameter variations over time were monitored at 15 minutes intervals by using custom-built gauges interfaced with a wireless data-logger system (Winet srl, Cesena, Italy); per each treatment, 12 well exposed fruit placed on 4 trees, on both sides of the canopy were simultaneously monitored following the methodology proposed by Lang (Lang, 1990). On each tree, three fruit with similar size were selected and treated as follows: one was left "intact" (normal vascular connections), one was "girdled" (phloem connection severed), and one was "detached" (with all vascular connections severed). This allowed the simultaneous calculation of vascular fluxes on 4 trees per treatment. Xylem and phloem flows were calculated using data from the three different conditions. This method assumes girdling doesn't affect xylem flow (van de Wal et al., 2017) and detachment doesn't affect transpiration. Besides, to date, this is the only method that allows estimation of vascular and transpiration flows in a field-short time scale and on a statistically sound number of samples.

The relative changes of fruit fresh weight in each time interval were calculated for each monitored fruit: intact (I), girdled (G), and detached (D). Then, xylem (X), phloem (P), and transpiration (T) flows were computed using the following equations. P=I- G; X = G - D; T =D. The values obtained from the monitored fruits were then used to calculate xylem and phloem flows. Daily cumulative fruit growth rate, phloem, xylem and transpiration flows were expressed both as weight changes per whole fruit (g fruit^{-1} day^{-1}) and per unit of fruit weight (g g^{-1} day^{-1}).

Sap flows

The Sakuratani method of thermal balance was used to calculate sap flow (Sakuratani, 1981). In order to compare the daily amount and patterns of the xylem sap flowing in the entire branch to the xylem flowing to the fruit, measurements were

made in conjunction with fruit gauge measurements (determined through the methodology described above).

Four sap flow sensors were installed in each period on branches with a diameter of 10 to 19 mm that were carrying at least one fruit on the same trees as the fruit gauges. A CR10X data logger (Campbell Scientific Inc., Logan, UT, USA) recorded data every minute from 3:30 a.m. to 10:30 p.m. and automatically averaged them every 15 minutes. The system operated continuously throughout each period of monitoring. The sap flow was then expressed as g m^2 h^{-1} after being normalized per unit of leaf area. Using ImageJ software (https://imagej.nih.gov/ij), the leaf area of each monitored branch was calculated by averaging the areas of six randomly selected leaves, and multiplying the result by the total number of leaves counted on the branch where the sap flow sensor was installed.

Fruit quality

At harvest, 30 fruits were collected for each treatment, and then their quality was analyzed in the laboratory. Fruit weight and diameter were measured with a digital scale and a caliper, respectively. To measure fruit flesh firmness, skin was removed on two different side of the fruit and then the analysis was carried out with a fruit texture analyzer equipped with a probe of 9 mm (FTA, GÜSS). Soluble solids content and acidity were measured with a digital brix-acidity meter provided by Atago. Dry matter content (%) was calculated as the ratio between fruit fresh weight and its own dry weight, obtained after a period in a fan-forced oven at 65°C. DA-index was measured thanks to a DA-meter (www.dameter.com).

Total phenolic content (TPC)

Samples for TPC analysis were harvested at 116 and 123 in 2021 and at 129 DAFB in 2022. Three fruit per plants were collected, for a total of 12 fruits per treatment. As soon as samples arrived in the lab, the skin and pulp were separated, frozen with liquid nitrogen, and kept at -80°C in a freezer. Later, the TPC was assessed using an adaptation of the Folin-Ciocalteau (Singleton et al., 1965) method proposed by Cantin et al (Cantín et al., 2009). In a tube, 1 g of frozen material was homogenized with an Ultraturrex for 2 minutes on ice using 10 mL of an extraction solution made up of

0.5N HCl in methanol/Milli-Qwater (80%v/v) and 80%v/v of water. The mixture was then incubated at 4 °C for an entire night before being centrifuged at 4 °C and 20000 g for 20 minutes the following day. The volume of the recovered supernatant was calculated. 0.5 µl of the extract were diluted with 0.5 µl of Folin-reagent Ciocalteu's in water. 1mL of 1N sodium carbonate (Na_2CO_3) was added after 3 minutes. After being mixed for 15 seconds, the tubes were kept at 20 °C in the dark for 60 minutes. Using a spectrophotometer, absorbance was measured at 725 nm. Gallic acid was used to prepare the standard calibration curves (3,4,5-trihydroxy- benzoic acid). Milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight were used to express the phenol content.

Statistical analyses

Averages and standard errors (SE) were computed for each day of measurement after all of the data that were taken into consideration were analyzed using a design that was entirely randomized. A Student's t-test was utilized to make cultivar comparisons on a date-by-date basis for each set of measurements.

Metabolomic analyses

Sample preparation. Part of the frozen material was lyophilized for fmetabolomic analysis. The metabolite extractions from 10 mg of lyophilized skin and flesh tissues was done adapting the protocol of Boutet et al. (2022). In brief, 1.5 mL of MeOH/Acetone/H2O/TriFluoroAcetic acid (30/42/28/0.1 v/v/v/v) and 500 ng of Apigenin (used as internal standard) were added to each sample which was then homogenized in 2-ml tubes using a FastPrep device (2 rounds x 0.30 sec, 14 000 rpm). The mixtures were then shaken for 30 min at 4°C using a ThermoMixerTM C (Eppendorf), placed in an ice-cooled ultrasonication bath for 2 min, and centrifuged for 1 min at the maximum speed to remove debris. The surnatant was then placed in a new tube, and the extraction procedure repeated for a second time adding 1 mL of extraction solution. The phases obtained were dried down in a SpeedVac vacuum concentrator and resuspended in 200 µl of water and ACN 10% and filtered.

LC-MS/MS analysis. Untargeted metabolomic data were acquired using a UHPLC system (Ultimate 3000 Thermo) coupled to quadrupole time of flight mass spectrometer (Q-Tof Impact II Bruker Daltonics, Bremen, Germany). For untargeted polar and semipolar metabolomic analysis, a Nucleoshell RP 18 plus reversed-phase column (2 9 100 mm, 2.7 lm; Macherey-Nagel) was used for chromatographic separation. The total run time for each sample was 35 min.

LC-MS/MS data processing. The data files (Bruker Daltonics, Bremen, Germany) obtained were first converted to .mzXML format using the MSConvert software (ProteoWizard package 3.0; Chambers et al., 2012). Data processing, mass detection, chromatogram building, deconvolution, isotope profiling, samples alignment and data export were performed using MZmine 2.53 software (http://mzmine.github.io/) for both positive and negative data files. The ADAP chromatogram builder (Myers et al., 2017) method was used with a minimum group size of scan 4, a group intensity threshold of 6000, a minimum highest intensity of 6500 and m/z tolerance of 8 ppm. Deconvolution was performed with the ADAP wavelets algorithm using the following setting: S/N threshold 8, peak duration range = 0.01–2 min, RT wavelet range 0.01–0.8 min, MS2 scan were paired using a m/z tolerance range of 0.15 Da and RT tolerance of 0.1 min. Then, isotopic peak grouper algorithm was used with a m/z tolerance of 8 ppm and RT tolerance of 0.1. All the peaks were filtered using feature list row filter keeping only peaks with MS2 scan. The alignment of samples was performed using the join aligner with an m/z tolerance of 8 ppm, a weight for m/z and RT at 1, a retention time tolerance of 0.1 min. Metabolites accumulation was normalized according to the quantity of internal standard (apigenin) and fruit tissue used for the extraction. A first research in the library with Mzmine was done, with identification module and 'custom database search' to begin the annotation with our library, currently containing 99 annotations (RT and m/z) in positive mode and 67 in negative mode, with RT tolerance of 0.3 min and m/z tolerance of 0.0025 Da or 6 ppm. This library research was repeated with the library containing references to the exact mass of 637 and 475 compounds in positive and negative mode, respectively. In addition, it has been used the identification module and "Adduct search", adding to the list the mass of H_2O.

Metabolomic network creation. Molecular networks were generated with MetGem software (Olivon et al., 2018; https://metgem.github.io) using the .mgf and .csv files obtained with MZmine2 analyses. The molecular network was optimized for the Positive Electro Spray Ionization (ESI+) and Negative Electro Spray Ionization (ESI-) datasets, and different cosine similarity score thresholds were tested. ESI- and ESI+ molecular networks were generated using cosine score thresholds from 0.7 to 0.9, depending on the samples analyzed. Molecular networks were exported to Cytoscape software (Shannon et al., 2003; https://cytoscape.org/) to format the metabolic categories.

Metabolite annotation of untargeted metabolomic data. Metabolite annotation was performed in four consecutive steps: 1) The obtained RT and m/z data of each feature were compared with INRAE homemade library containing more than 150 standards or experimental common features (RT, m/z); 2) The ESI- and ESI+ metabolomic data used for molecular network analyses were searched against the available MS2 spectral libraries (Massbank NA, GNPS Public Spectral Library, NIST14 Tandem, NIH Natural Product and MS-Dial), with absolute m/z tolerance of 0.02, 4 minimum matched peaks and minimal cosine score of 0.8; 3) Not-annotated metabolites that belong to molecular network clusters containing annotated metabolites from steps 1 and 2 were assigned to the same chemical family; 4) For metabolites that had no or unclear annotation, Sirius software (https://bio.informatik.uni-jena.de/software/sirius/) was used. Sirius is based on machine learning techniques that use available chemical structures and MS/MS data from chemical databanks to propose structures of unknown compounds.

Statistical analyses and enrichment analyses. To assess statistical differences in metabolite concentration in fruit from plants that received different irrigation amount, a volcano plot was done using the Metaboanalyst software (MetaboAnalyst). Enrichment analysis was done by using a hypergeometric test, and the FDR was controlled across all the treatments at level 0.05. The metabolite reference set was defined from the annotated and unknown metabolic categories. The analyses were performed with the software R. The contrasts and the enrichment tests were written according to the functions of the tool DiCoExpress (Lambert et al., 2020).

RESULTS

Water relations

The Ψ_{stem} measured before irrigation stop in 2021 showed comparable values between treatments. Then, until harvest the season was characterized by lower Ψ_{stem} values in the stress treatment, reaching at harvest values of -0.73 MPa and -1.01 MPa in Control and Stress trees, respectively (Figure 1a). Instead, the seasonal pattern of Ψ_{stem} during 2022 presented similar values regardless of the treatment (Figure 1b). Looking at the seasonal pattern of Ψ_{leaf} in 2021 no differences were observed. However, Ψ_{leaf} in Stress trees reached values of -2 MPa while Control was -1.5 MPa at harvest 2022 (Figure 1d) .

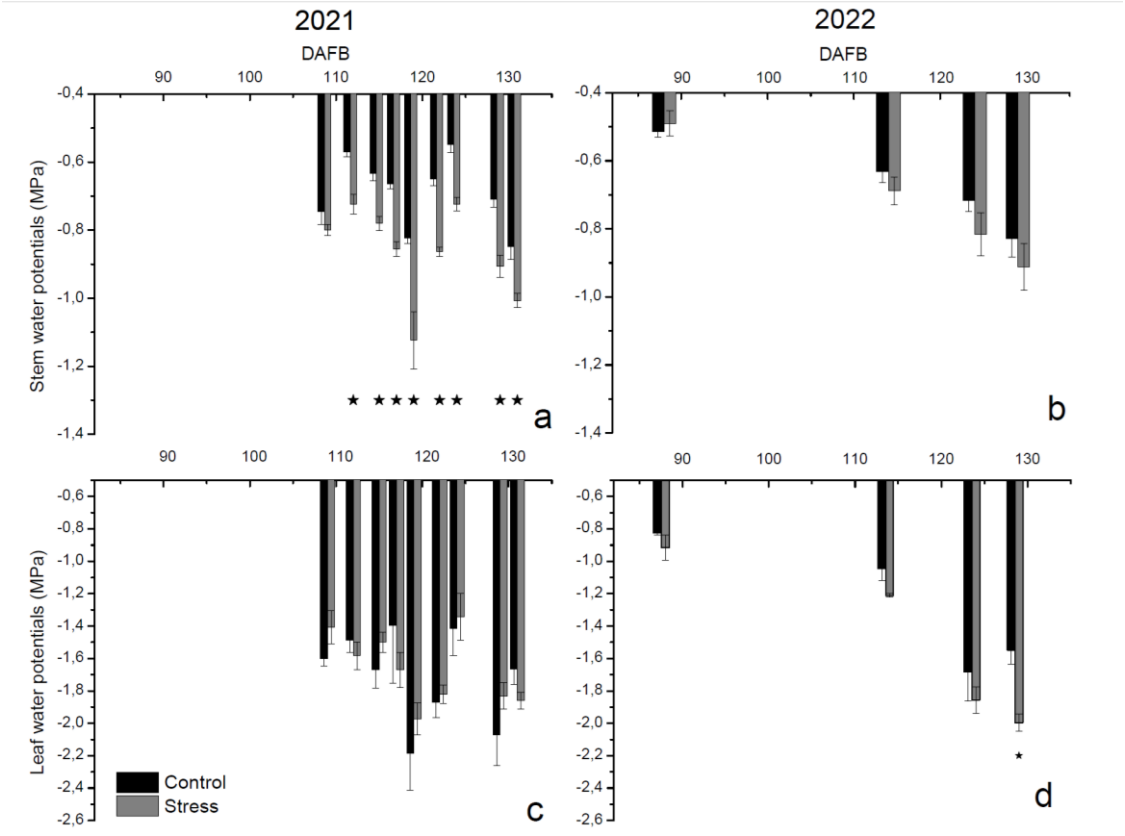

Figure 1. Seasonal pattern of stem (a, b) and leaf (b, d) water potentials in Control (black bars) and Stress (grey bars) treatments in 2021 and 2022. Measurements were carried out at 109,112, 115, 117, 119, 122, 124, 129 and 131 DAFB in 2021 and at 88, 114, 124 and 129 DAFB in 2022. Stars (*) indicates statistically significant difference between treatments ($p<0.05$).

Leaf gas exchanges

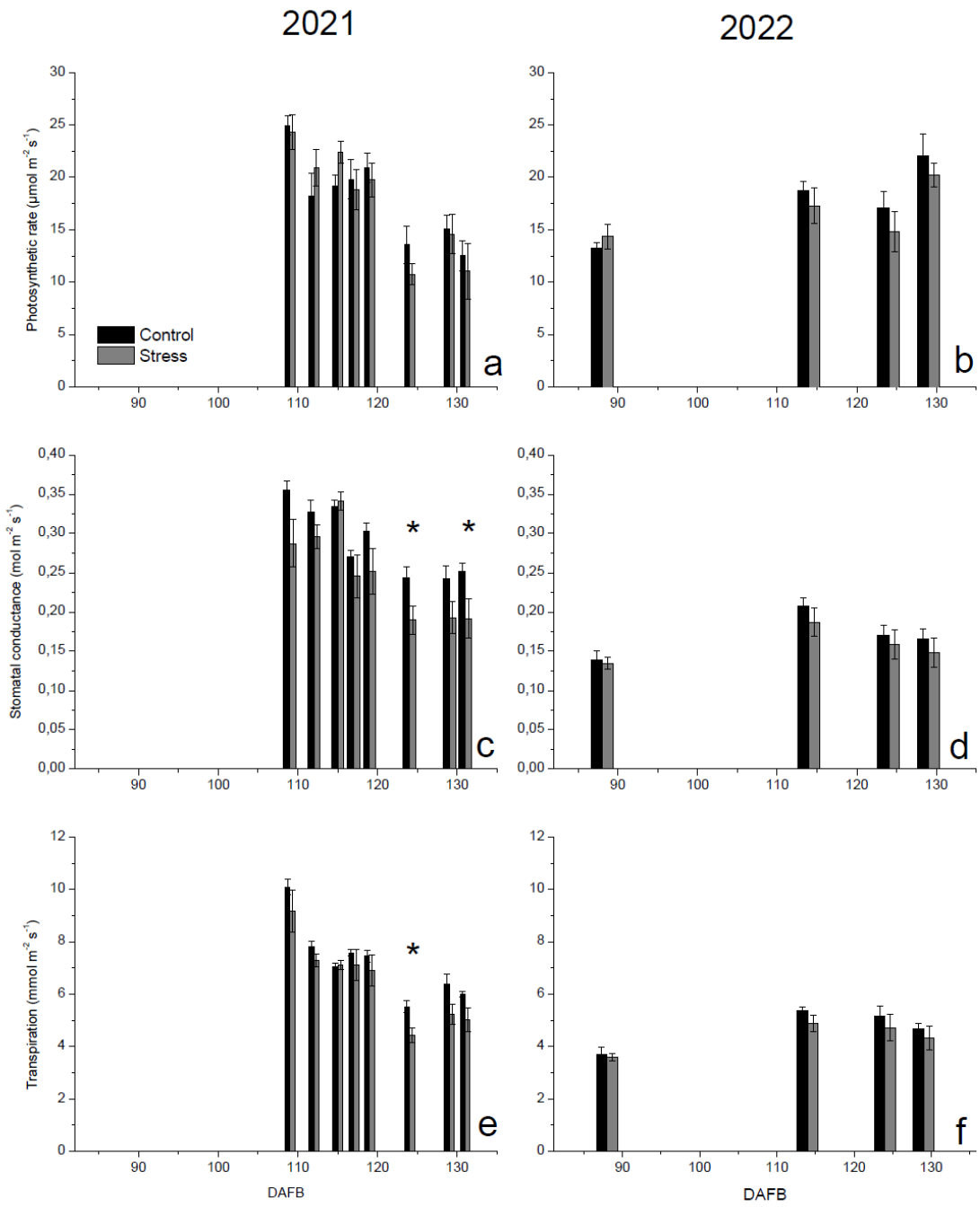

Figure 2. Seasonal photosynthetic (a, b) and transpiration (c, d) rates and stomatal conductance (e, f) in Control (black bar) and Stress (grey bar) treatments in 2021 and 2022. Measurements were carried out at 109,112, 115, 117, 119, 122, 124, 129 and 131 DAFB in 2021 and at 88, 114, 124 and 129 DAFB in 2022. Stars (*) indicates statistically significant difference between treatments ($p<0.05$).

In both years, the photosynthetic rate was not affected by the irrigation treatment applied (Figure 2a, 2b). Stomatal conductance was found to be lower ($p<0.05$) in Stress treatment, with values of 0.19 mol m^{-2} s^{-1} (in both dates) against Control that was 0.24 mol m^{-2} s^{-1} and of 0.25 mol m^{-2} s^{-1} at 124 DAFB and 131 DAFB 2021, respectively. However, no differences in stomatal conductance were observed in 2022 (Figure 2c, 2d). Leaf transpiration in 2021 showed a decreasing trend along the season. Stress trees showed values of 5.5 mmol m^{-2} s^{-1} against 4.4 mmol m^{-2} s^{-1} of Control trees at 124 DAFB. However, in 2022 the irrigation treatment did not influence transpiration values, which remained almost constant along the season (Figure 2e, 2f).

Fruit and shoot seasonal growth

In general, seasonal fruit growth in 2022 showed a similar pattern, with no differences in final fruit size (Figure 3a). The seasonal absolute growth rate as well followed a similar trend, with no significant differences. It can be noted that the highest growth rates were at 114 DAFB in Stress and at 124 DAFB in Control (Figure 3b).

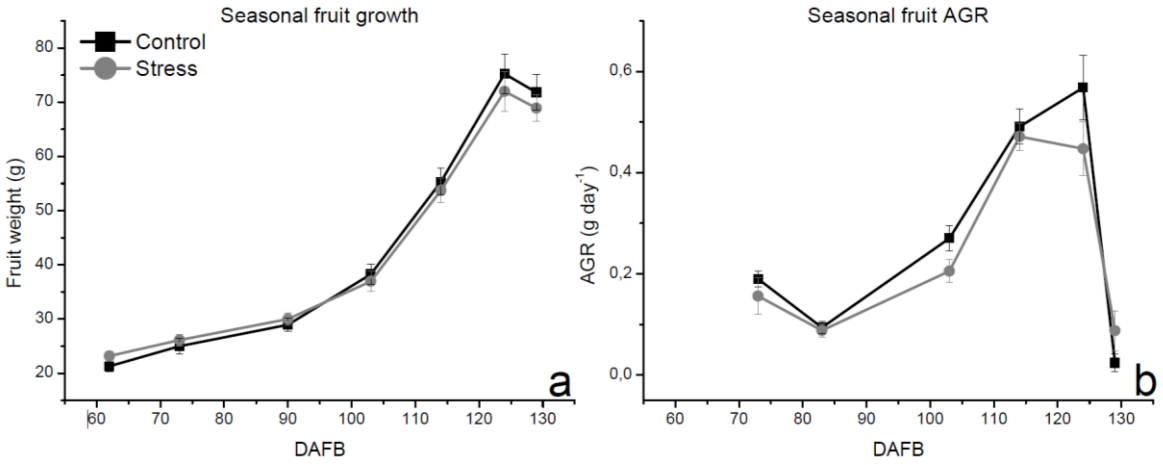

Figure 3. Seasonal fruit growth (a) and AGR (b) in Control (black lines) and Stress (grey lines) trees in 2022. Stars (*) represent statistical significant differences at $p<0.05$ resulted by t-test.

Seasonal shoot growth presented a steady increase along the whole season, with similar values in both treatments. At harvest, the shoot length measured was of 53 cm and 44 cm in Control and Stress trees, respectively. In both treatments sampled shoots presented high variability, with a standard error of 11 cm and 9 cm in Control and Stress, respectively (Figure 4a). Shoot AGR seemed to be unaffected by the irrigation treatment applied, reaching the lowest values at 124 DAFB and then increasing again towards harvest (Figure 4b).

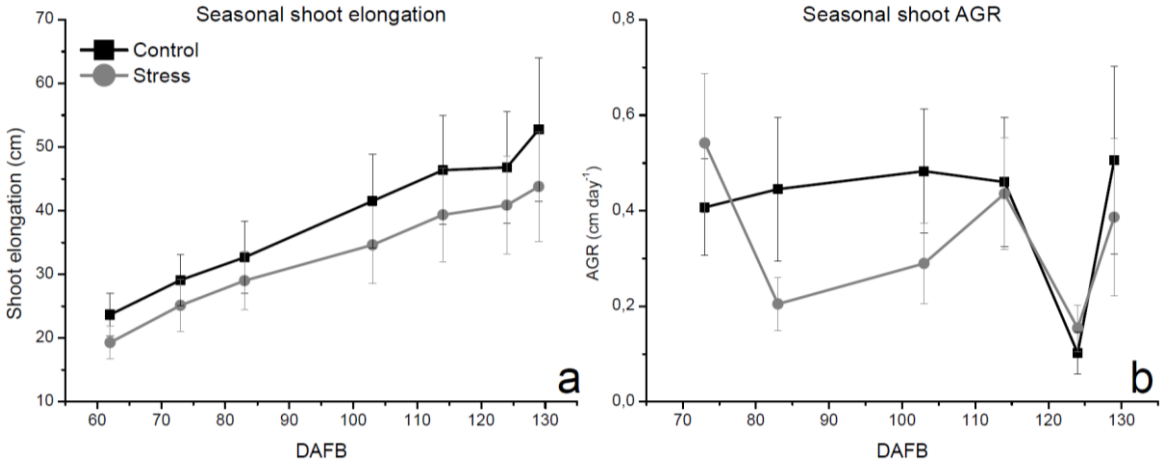

Figure 4. Seasonal shoot elongation (a) and AGR (b) in Control (black lines) and Stress (grey lines) in 2022. Stars (*) represent statistically significant differences at $p<0.05$ resulted by t-test.

Seasonal vascular and transpiration fluxes to/from the fruit

Specific fruit vascular flows (g g-1 day-1) showed higher values in Stress treatment at 115 DAFB 2021 for transpiration rates (-0.06 g g^{-1} day^{-1} and -0.11 g g^{-1} day^{-1} in control and stress, respectively) and xylem flows (0.05 g g^{-1} day^{-1} in control and 0.10 g g^{-1} day^{-1} in stress treatment, respectively). However, from 117 DAFB 2021 until harvest 2021 no differences were observed (Figure 5a, c, e, f). Considering the same vascular flows in absolute values (g day^{-1}) at 115 DAFB 2021 Stress fruit showed higher xylem inflow (9.25 g day^{-1}) compared to controls (4.10 g day^{-1}) (Fig. 5b, d, f, h).

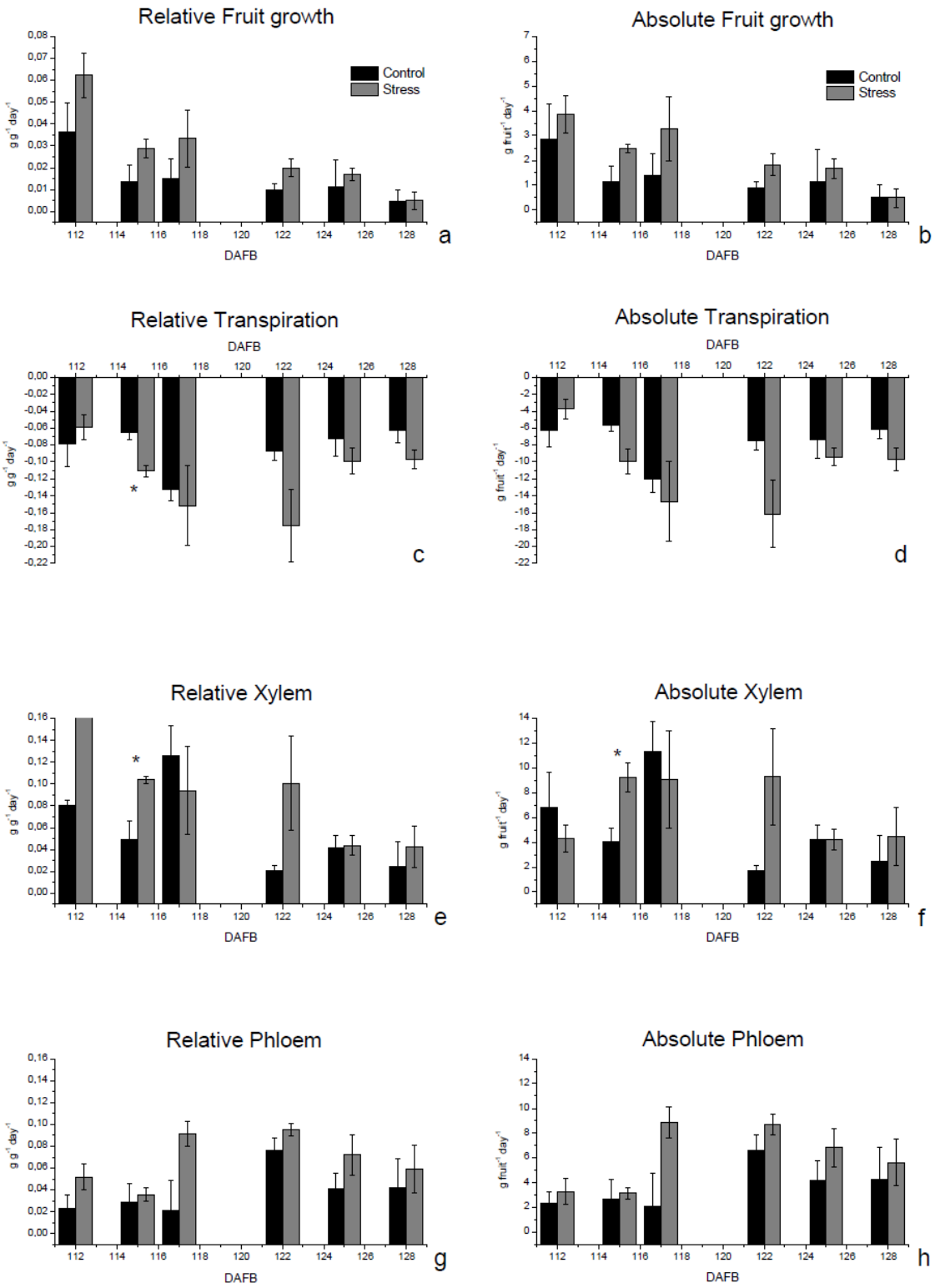

Figure 5. Mean (+ SE) of the relative (RGR) (a) and absolute (AGR) (b) growth rates, specific (c) and whole fruit (d) transpiration, specific (e) and whole fruit (f) xylem flow, specific (g) and whole fruit (h) phloem flow at 112, 115, 117, 122, 125, and 128 DAFB 2021.

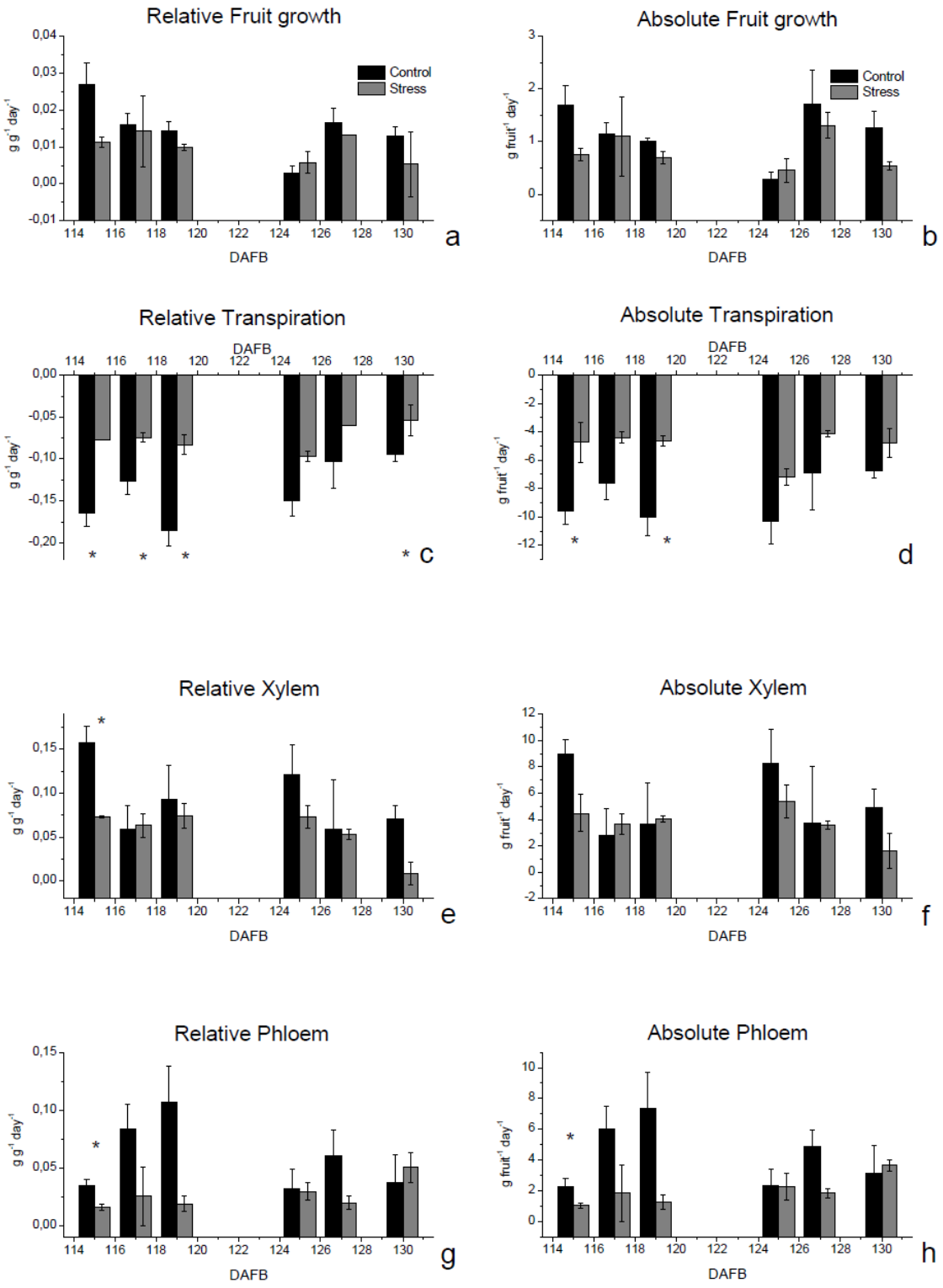

Figure 6. Mean (+ SE) of the relative (RGR) (a) and absolute (AGR) (b) growth rates, specific (c) and whole fruit (d) transpiration, specific (e) and whole fruit (f) xylem flow, specific (g) and whole fruit (h) phloem flow at 115, 117, 119, 125, 127 and 131 DAFB 2022.

The trial carried out in 2022 showed similar values for fruit growth, while relative transpiration was reduced in Stress fruit at 115, 117, 119 and 131 DAFB 2022. Looking at the absolute transpiration rates, Stress fruit showed values ($p<0.05$) of -0.07 g g^{-1} day^{-1} and -0.08 g g^{-1} day^{-1} against -0.16 g g^{-1} day^{-1} and -0.18 g g^{-1} day^{-1} of Control trees at 115 and 119 DAFB 2022, respectively. Specific xylem (0.07 g g^{-1} day^{-1} and 0.15 g g^{-1} day^{-1} in Stress and Control, respectively) and phloem (0.01 g g^{-1} day^{-1} in Stress and 0.03 g g^{-1} day^{-1} in Control) flows registered a decreased flux in Stress fruit at 115 DAFB 2022 (Figure 6).

Daily patterns of fruit growth, transpiration and vascular fluxes

Specific daily fruit growth rate showed higher rates in Stress between midnight and 4 a.m. at 115 and 122 DAFB 2021. In fact, Stress fruit reached higher growth rates with values of 0.06 mg g^{-1} min^{-1} against 0.02 mg g^{-1} min^{-1} of the Control at 115 DAFB 2021 and of 0.05 mg g^{-1} min^{-1} 0.04 mg g^{-1} min^{-1} in Stress and Control, respectively, at 122 DAFB 2021. At 128 DAFB 2021 it was found a higher growth rate of 0.001 mg g^{-1} min^{-1} in Stress while Control fruit presented values of 0.0009 mg g^{-1} min^{-1} at 8 p.m. (Figure 7). In 2022 the daily specific growth rates presented a more similar pattern regardless of the treatment applied. As a matter of fact, Stress fruit presented higher values at 3 p.m. at 115 DAFB 2022 and at 7 p.m. at 130 DAFB 2022. Control fruit showed a height growth rate of 0.06 mg g^{-1} min^{-1} against 0.02 mg g^{-1} min^{-1} of Stress, at 11 p.m. at 115 DAFB 2022 (Figure 6).

Fruit daily transpiration rate presented different pattern according to the year considered. In 2021, Stress fruit presented higher transpiration from 9 a.m. to 3 p.m. at 115 DAFB 2021 and at 4 a.m. and 5 p.m. at 128 DAFB 2021. The opposite trend was seen in 2022 in which it was Control fruit in presenting the highest transpiration rates from midnight to 1 a.m. and from midday to 11 p.m. at 115 DAFB 2022. In addition, higher values were registered in Control fruit also at 125 DAFB at 11 a.m., from 4 p.m. to 6p.m., at 9 p.m. and at 11 p.m.. At 130 DAFB 2022 no differences were recorded (Figure 8).

Xylem daily flows in 2021 were found to be higher in Stress fruit at 6 a.m., from 5 p.m. to 7 p.m. and from 10 p.m. to 11 p.m.. However, at 122 and 128 DAFB irrigation

treatment seemed to not influence xylem flows, since no differences were recorded (Figure 7). As for transpiration, the daily pattern showed in 2022 is opposite to the one seen in 2021. At 115 DAFB 2022, in fact, the highest flow is the one in Control fruit, from 1 p.m. to 8 p.m.. On the other days no differences were registered (Figure 8).

Phloem flows presented different pattern based on the day considered. At 115 DAFB 2021 the Stress fruit presented the higher flux from 10 p.m. to midnight. At 122 DAFB 2021 it was Stress fruit presenting the higher values of phloem influx from midnight to 3 a.m. and at 9 a.m.. However, at 128 DAFB 2021 no differences were found (Figure 7). In 2022, Control fruit presented higher values from midnight to 2 a.m., at 5 a.m., from 7 a.m. to 8 a.m., from 3 p.m. to 4 p.m. and at 11 p.m.,. On the other days, both treatment presented a similar pattern (Figure 8).

Sap flows

In general, sap flow was not affected by the lack of irrigation. In fact, during the three monitoring days at 115, 122 and 128 DAFB 2021 no differences were found. Stress treatment followed the same daily pattern of Control trees, presenting sometimes slightly higher values especially in the late evening (Figure 9).

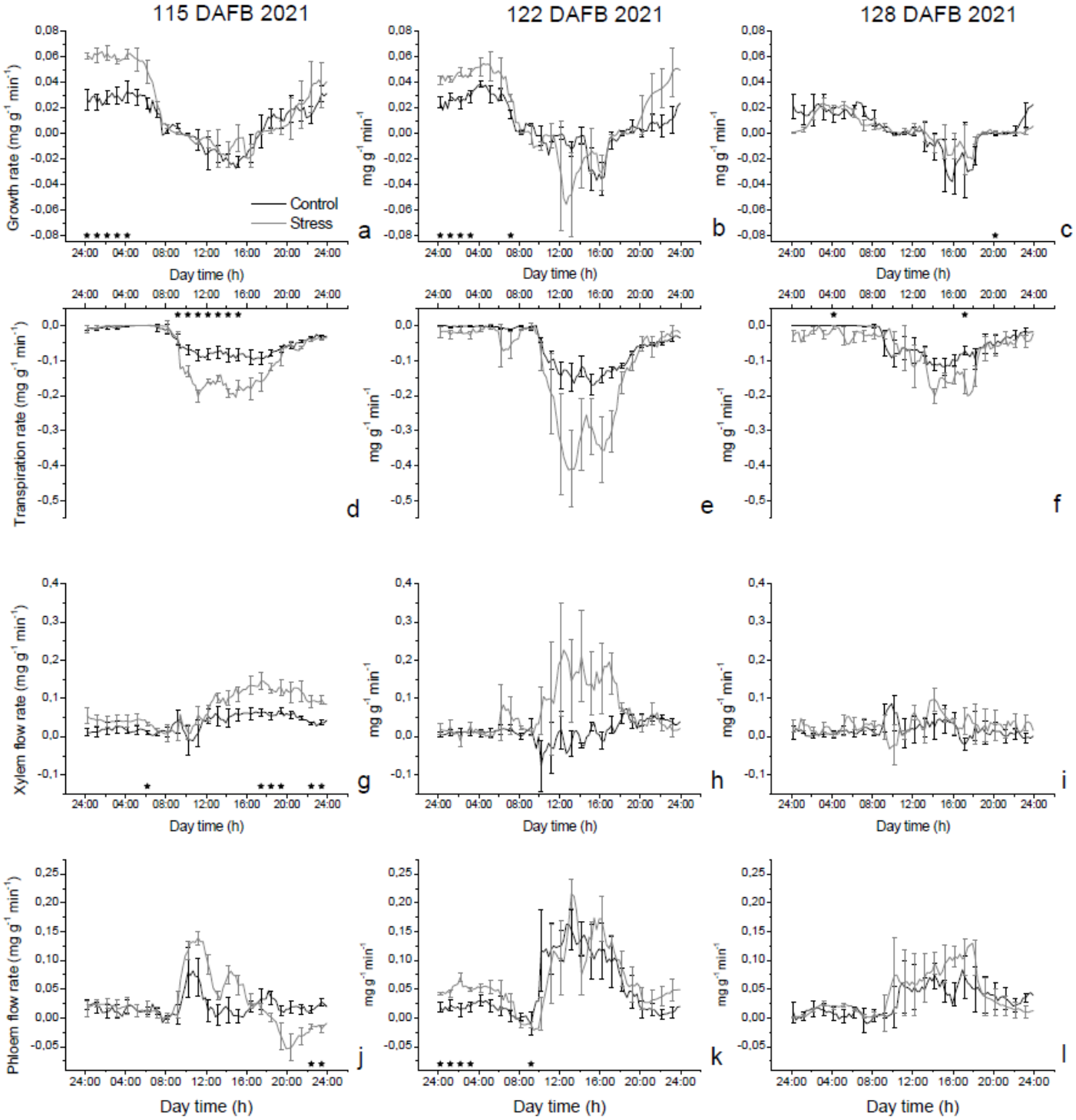

Figure 7. Average diurnal courses of fruit relative growth rate (RGR) and specific phloem, xylem, transpiration flow rates (mg g-1min-1) at 115 (a,d,g,j), 122 (b,e,h,k), and 128 (c,f,i,l) DAFB in 2021. For each parameter, data are the means of 4 replicates. Maximum SEs for fruit growth were 10,76, 20,49, 35,05 and 74,71. Maximum SEs for transpiration were 0,122, 0,114, 0,028 and 0,068 at 57, 81, 111 and 129 DAFB, respectively. Maximum SEs for xylem were 0,131, 0,147, 0,030 and 0,061. Maximum SEs for phloem were 0,085, 0,072, 0,044 and 0,045.

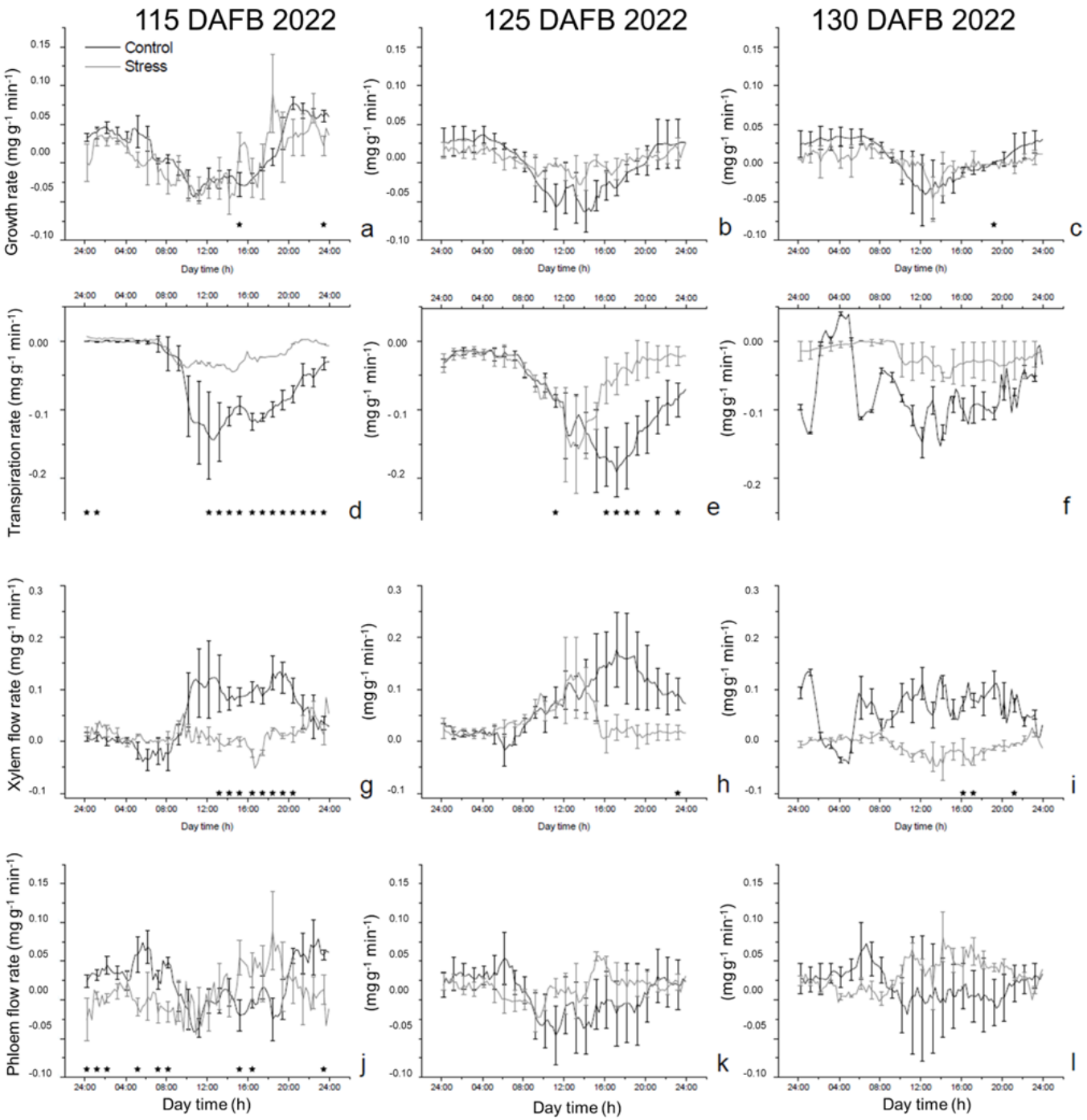

Figure 8. Average diurnal courses of fruit relative growth rate (RGR) and specific phloem, xylem, transpiration flow rates (mg g-1min-1) at 115 (a,d,g,j), 125 (b,e,h,k), and 130 (c,f,i,l) DAFB in 2022. For each parameter, data are the means of 4 replicates. Maximum SEs for fruit growth were 10,76, 20,49, 35,05 and 74,71. Maximum SEs for transpiration were 0,122, 0,114, 0,028 and 0,068 at 57, 81, 111 and 129 DAFB, respectively. Maximum SEs for xylem were 0,131, 0,147, 0,030 and 0,061. Maximum SEs for phloem were 0,085, 0,072, 0,044 and 0,045.

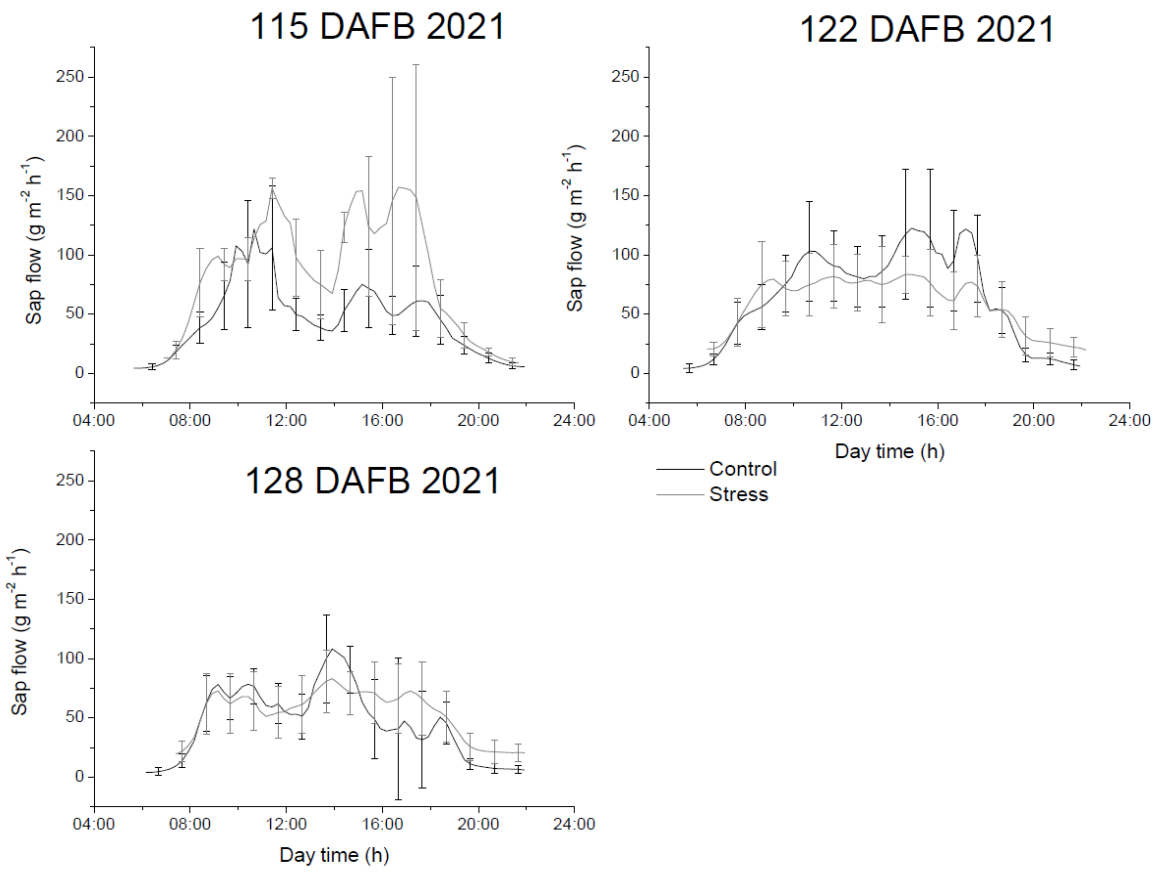

Figure 9. Diurnal courses of sap flow rate (g m^{-2} h^{-1}) at 115 (a), 122 (b), 128 (c) DAFB 2021 and VPD (kPa) on the three days considered (d). For each parameter, data are the means of 4 replicates. Maximum SEs for sap flow were 10,76, 20,49, 35,05 and 74,71.

Among the fruit quality traits measured at harvest (Table 1), the chlorophyll degradation level was the only parameter showing statistically significant differences between the two treatments in 2021. The mean values obtained in the control was of 0.12 and in the stress was of 0.19. In 2022, fruit soluble solids content was higher in Stress fruit with values of 13°brix against 12°brix of the control. Fruit weight presented statistically significant lower values in Stress treatment (78.5 g and 83.4 g in Stress and Control, respectively).

Flesh firmness and dry matter content showed similar values between treatments, with no significant differences.

Table 1. Fruit quality parameters in apricot at harvest 2021 and 2022. Each value is the mean of at least 30 fruit. Treatments were compared by a student's t test. Stars indicate statistical difference at p>0.05.

		Fresh weight (g)	Diameter (mm)	Flesh firmness (kg/cm^2)	SSC (° Brix)	Dry matter (%)	DA-meter
2021	**Control**	106 ± 3.8	52.3 ± 0.7	2.5 ± 0.2	12.7 ± 0.5	12.6 ± 1.1	0.12 ± 0.02
	Stress	104.5 ± 4.1	52.7 ± 0.7	2.8 ± 0.2	12.3 ± 0.7	10.1 ± 0.9	0.19 ± 0.02
	Sign.	n/s	n/s	n/s	n/s	n/s	*
2022	**Control**	83.4 ± 1.7	48.3 ± 0.4	3.6 ± 0.2	12 ± 0.3	10.8 ± 0.3	
	Stress	78.5 ± 1.7	48.2 ± 0.4	3.3 ± 0.2	13 ± 0.4	11.4 ± 0.3	
	Sign.	*	n/s	n/s	*	n/s	

Total phenolic content (TPC)

Table 2. Total phenolic content in apricot flesh and skin tissues, in 2021. Each values is the mean of at least 4 replicates. Treatments were compared by a Student's t test. No statistical differences were highlighted

	Flesh TPC (mg of gallic acid/g of fruit flesh	
	116 DAFB	123 DAFB
Control	**1.9** ± 0.25	**0.32** ± 0.07
Stressed	**2.3** ± 0.16	**1** ± 0.3

It can be seen that total phenolic content at the first sampling date showed higher values than at the second sampling, that was at harvest 2021. At 116 DAFB Stress fruit presented values of 2.3 mg of gallic acid /g of fruit tissue while at harvest Stress fruit presented values three-times higher than Control but without being significantly different (Table 2).

Metabolomic analyses

The profiling of specialized metabolites (SM) of apricot revealed a wide range of variability (Figure 10 and 11). The discovery of 1493 and 1671 peaks of putative known and unknown metabolites [metabolic features (Mfs)] in the fruit skin and flesh, respectively, was made possible by employing untargeted metabolomic data using Electro Spray Ionization in both positive (ESI+) and negative (ESI-) modes. Using the MetGem tool, a molecular network was built using the LC-MS/MS data (Figure 10 and 11). This method enables the discovery of clusters of unknown metabolites by using the MS/MS information for annotated compounds to cluster

molecules that have a high degree of structural similarity based on their MS2 spectra (Olivon et al., 2018). The analysis of apricot metabolites was able to categorize 24% and 28% of the discovered compounds to a metabolic category in skin and flesh, respectively, by using public databases for annotation and internal standards in conjunction with a molecular network. In contrast, 25% (in the skin) and 14% (in the flesh) of the metabolites were discovered to be included in clusters that could not be linked to a metabolic class, while 57% and 51% of the metabolites were not part of any cluster and were not annotated in the skin and flesh, respectively (Fig 10 and 11). Overall, SMs identified in apricot fruit have been assigned to 20 (in the skin) and 14 (in the flesh) putative metabolic categories. The SMs present in both tissues were: aminoacids, blumenols, carbohydrates, carboxylic acids, cinnamic acids, cyanides, flavonols, falvan-3-ols, flavones, flavanones, lipids and fatty acids, lignans, phenols and terpenoids. The compounds present only in the skin were the following: benzoyl derivates, disaccharides, glycosides, nucleosides, saccharolipids and flavonoids.

SMs pattern was investigated at two different timings in 2021: at 116 DAFB and at harvest (123 DAFB). At the first sampling 87 and 151 upregulated metabolites were found in the skin and the flesh of Stress fruit, respectively, while the ones downregulated in Stress fruit were 3 in the skin and 4 in the flesh. The major upregulated compounds belonged to terpenoids, carbohydrates and unknown clusters in the flesh and to cinnamic acids, terpenoids and carbohydrates in the skin. Probably the carbohydrates found to be both up- and down-regulated belonged to different categories, but unfortunately it was not possible to determine their structure. Another possible scenario could be that the molecules identified as carbohydrates were bounded to other specialized metabololites through glycosylation, but it was recognized only the main carbohydrate molecules. The results found for the second sampling date showed however a decrease in the upregulated metabolites and an increase in the downregulated ones. In fact, SMs upregulated in Stress fruit were 3 in the skin and 6 in the flesh while the downregulated were 209 and 12 in skin and flesh, respectively.

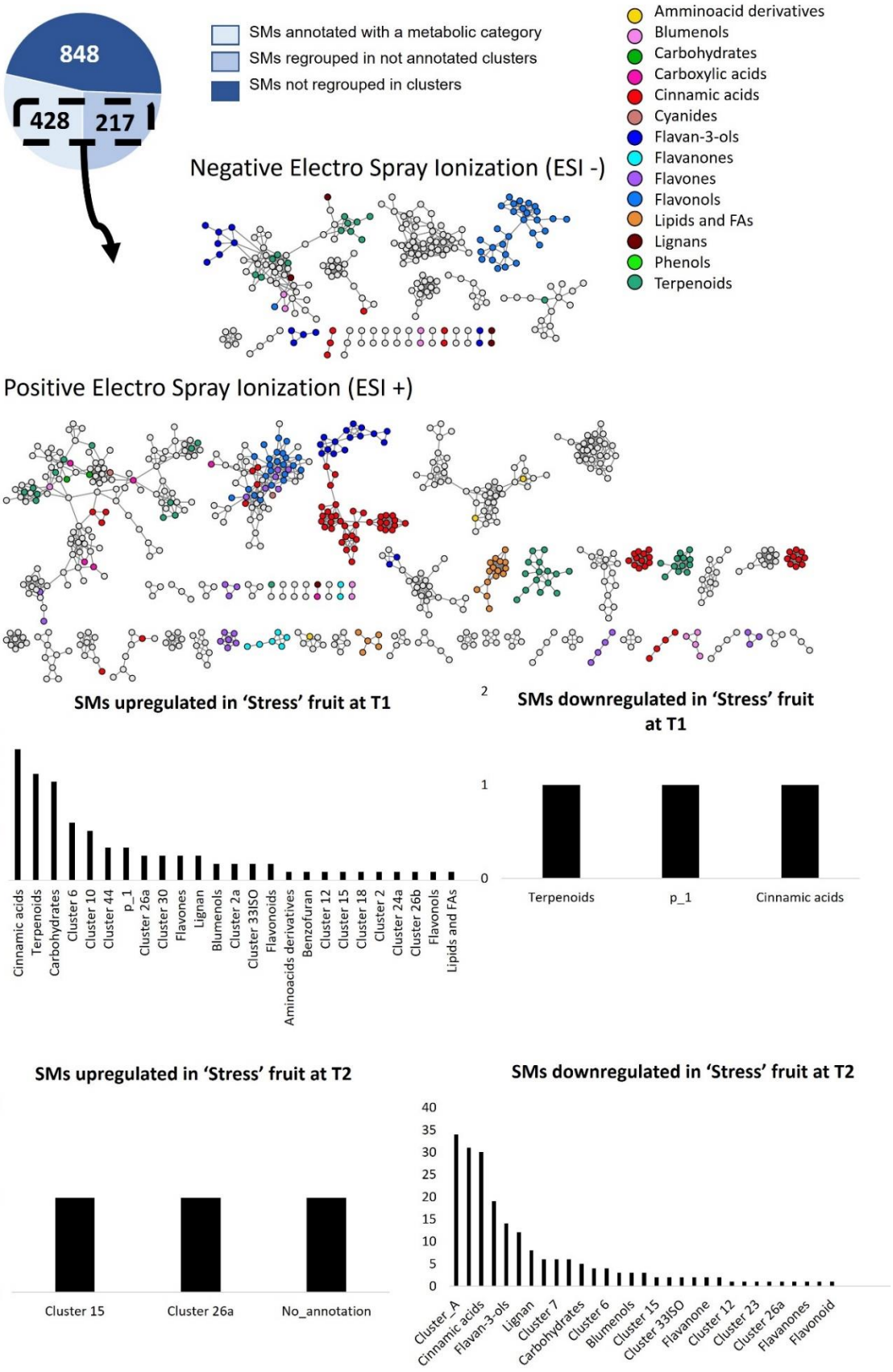

Figure 10. Molecular networks and annotation of the skin-specialized metabolites (SMs). (a) Numbers of SMs present in a molecular network with or without an annotation. (b) The molecular networks are shown for metabolite analyses carried out in positive (ESI+) and negative (ESI-) ionization modes. Different colours correspond to different metabolic classes. Metabolites are grouped based on their chemical structures. Cosine similarity scores of 0.8 and 0.75 were used for ESI+ and ESI-, respectively. (c) Histograms showing the upregulated and downregulated metabolic categories identified in apricot skin at T1 and T2.

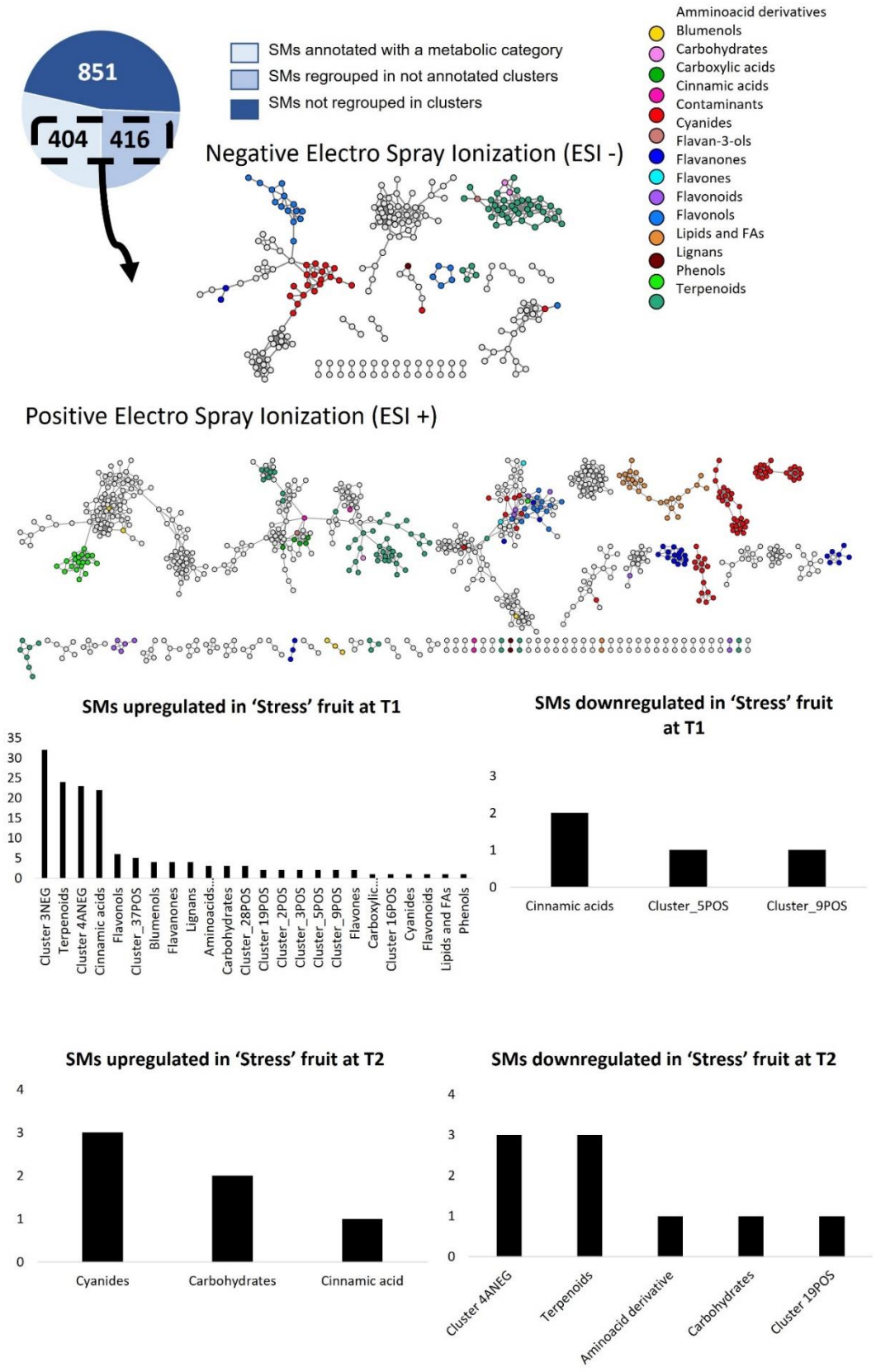

Figure 11. Molecular networks and annotation of the flesh-specialized metabolites (SMs). (a) Numbers of SMs present in a molecular network with or without an annotation. (b) The molecular networks are shown for metabolite analyses carried out in positive (ESI+) and negative (ESI-) ionization modes. Different colours correspond to different metabolic classes. Metabolites are grouped based on their chemical structures. Cosine similarity scores of 0.8 and 0.75 were used for ESI+ and ESI-, respectively. (c) Histograms showing the upregulated and downregulated metabolic categories identified in apricot flesh at T1 and T2.

DISCUSSION

Periods of water scarcity seem to have impacted apricot plant physiology only in a slight way. In fact, although in 2021 stem water potential in Stress trees exhibited a slight drop after the beginning of the trial until harvest, the other physiological parameters continued to present similar values to those of Control plants. This confirms the importance of monitoring stem water potential since it is a parameter that quickly responds when water availability decreases (Shackel, 2007). However, during the season 2022 no differences were recorded in stem water potential, even though the irrigation was closed 25 days earlier than in 2021 thus leading to an impressive long period of 47 days without irrigation. This can be due to a modification of the plant conductive system occurred in the previous year that helped the plant adapting to subsequent water stress periods. According to literature, plants can be able to modify their stress response on the basis of past stress events, using the so-called "ecological stress memory" (Bruce et al., 2007). It was observed how drought stress in forest trees can lead to the formation of reduced xylem vessels decreasing the risk of embolism in the long-term (Pretzsch, 2021). This adaptation strategy is probably present also in fruit tree crops since they share some common morphological characteristics with silvicultural crops. At the moment, some evidences on the cross-priming positive effect on following drought stress are present in olive (Ben Abdallah et al., 2022) as well as stress memory transmission in citrus buds (de Oliveira Sousa et al., 2022). Leaf photosynthetic rate in both years was not affected by the lack of irrigation while stomatal conductance was affected at 124 and 131 DAFB 2021, while transpiration was lower in Stress trees at 131 DAFB 2021 (Figure 2). Considering that stomatal conductance was reduced towards harvest in order to minimize water losses, it can be considered as an adaptive response to drought stress as previous findings (Ruiz-Sánchez et al., 2007, 2000; Torrecillas et al., 1999). However, in 2022 no differences were recorded, supporting the hypothesis of stress memory above mentioned. Seasonal fruit grow showed a double sigmoid pattern typical of stone fruit, with similar values in both treatments considered (Figure 3). According to literature, fruit growth in apricot is sensitive to water reduction higher than the 50% of the crop evapotranspiration rates, when coupled to reduced shoot growth (Pérez-Pastor et al., 2014, 2009). Considering that seasonal

shoot elongation pattern was similar in both treatments, it can be supposed that carbohydrates production and partitioning was sufficient to sustain fruit growth even though water availability was decrease for fruit at growth stage III (Figure 4). Thanks to previous studies it is known that apricot fruit is characterized by high transpiration rates also close to harvest, while the xylem and phloem flows tend to decrease and increase, respectively towards harvest (Giovannini et al., 2022). Cumulated fruit vascular fluxes showed two different patterns according to the year considered (Figure 5 and 6). In 2021 Stress fruit showed higher values of specific xylem flows at 115 DAFB 2021, after 6 days since the irrigation closure. Instead, in 2022, specific transpiration, phloem and xylem flows were higher in Control fruit at 115 DAFB 2022. This could be attributed to the fruit variability but also to the different crop load levels in the two years. In fact a higher crop load was present in 2022 due to the lack of late frost. A higher number of fruit might have lead to a higher competition and thus to a stronger susceptibility to reduced water availability. Because of the larger VPDs, fruit that were more exposed to light would have transpired more. These differences, however, were not found later in the season and at harvest. In the last two weeks before harvest, xylem and phloem fruit inflows reduced slightly their amounts, presenting however higher values than those found in pear (Morandi et al., 2014).

On a daily scale, fruit specific vascular flows reported different trends in 2021 and 2022. In general, fruit vascular flows in Stress treatment presented higher values in the late evening or during the night in 2021 (Figure 7 and 8). However, these differences were not found in 2022 probably because influenced by the different seasonal pattern. Sap flow in the branch showed comparable values in plants subjected to different irrigation treatments. This means that the same amount of water was transported to leaves and fruit, that were able to maintain elevated transpiration rates both the fruit and the leaves. The apricot physiological response and adaptation to water shortages led to fruit with similar quality characteristics (Table 1). In 2021 the chlorophyll degradation was the only parameter to present lower values in the Stress treatment meaning that fruit ripening was enhanced in Stress fruit. This was found also in other researches (Torrecillas et al., 2000). Soluble solids content showed higher values in fruit from plants subjected to drought stress

in 2022. This confirms what previously found on other studies on RDI on apricot (Perez-Sarmiento et al., 2010). In apricot, xylem flow maintains high values even towards harvest thanks to relatively high transpiration values (Giovannini et al., 2022). However, fruit from Stress plants may have suffered a slight status of dehydration (although not translated in decrease of fruit size and weight), leading to higher SSC. Fruit flesh TPC showed a marked increase after one week from the irrigation stop in 2021, with values almost doubled in Stress against Control (Table 2). However, fruit skin usually contains more polyphenols, reaching up to 10 times higher values, in agreement with other studies on plum, peach, and apricot (Veberic and Stampar, 2005). This is because they protect against UV radiation, pathogens, and environmental stress (Manach et al 2004). We discovered a large diversity of SM classes (20 in total) in apricot fruit thanks to the molecular network approach (Elie et al., 2019; Olivon et al., 2018) combined with manual confirmation of metabolite annotation using molecular structure identification (Duhrkop et al., 2019) (Figure 10 and 11). The current findings revealed that 29% (in the skin) and 24% (in the flesh) of the identified metabolites are annotated or belong to a cluster with annotated metabolites. The metabolites present in public databases used for annotation account for a small portion of the plant metabolome (13% - 21%). It has to be noted that no carotenoids were identified with LC/MS-MS, probably because the method extraction was not adapted for lipophilic substances. The lack of irrigation has induced modifications in the quantity of some metabolites, with 247 and 226 up- and down-regulated features in skin and flesh, respectively.

CONCLUSION

In our conditions, apricot trees were able to afford long period in absence of irrigation without showing significant decrease in plant physiology and fruit growth over two years of study. This might be due to a possible modification in the conductive woody tissues but for sure the use of vigorous rootstocks characterized with deep root system enhanced the plant resilience to water stress. This phenomenon might be defined as tree “stress memory”, but further researches are needed to deeply investigate and understand this aspect.

In addition, deficit irrigation seems to improve fruit quality in terms of sugar content but the results shown in this study unfortunately do not provide strong demonstration of this relation. In conclusion, the irrigation scheduling in apricot is surely overestimated in the actual decision support system available in Emilia-Romagna region. This study is the first attempt to link plant physiological performances under irrigation shortages to fruit metabolomic composition. Although this new knowledge needs to be further investigated, it could represent the starting point for a new approach of sustainable and resilient fruit production, based not only on fruit size but rather on fruit nutraceutical traits.

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CHAPTER V

CONCLUSIONS

The objectives of these studies were to test the effect of deficit irrigation in different fruit tree crops, monitoring the physiological repercussion both on the plant physiological behaviour and on the fruit, especially in terms of fruit nutraceutical quality.

On apple (Chapter II), plants that were subjected to a reduced deficit irrigation (50% of Etc) showed a decrease in stem water potential values, leading to a decrease in final fruit size at harvest during the first year of trial. Thanks to the daily anticipation in vascular flows and to a reduction in transpiration fruit subjected to deficit irrigation were able to maintain similar growth rates to control trees. Moreover, the comparison of fruit vascular flows at 107 DAFB in two different years showed that the timing of xylem disfunction during the season depends on the seasonal weather conditions, capable to anticipate or delay the xylem vessel functionality. Fruit total phenolic content was not influenced by reduced irrigation, probably because the stress imposed was not sufficient to activate an up-regulation in the synthesis of specialized metabolites.

On sour cherry (Chapter III), plants coped with water deficit without showing particularly intense deficiency and without compromising their physiological functions. Moreover, fruit soluble sugars content was not affected by the irrigation treatment applied while total phenolic content was increased in the non-irrigated trees. Water stress deeply modified the metabolomic landscape of spur cherry fruit with an increase in lipids and carbohydrates as well as a drecrease in cinnamic and carboxylic acid and terpenoid metabolites. For these reasons, the lack of irrigation in the last weeks before haverst can be successfully applied on sour cherry, without penalizing plant performances and could also be used as a tool to modulate the fruit metabolome.

On apricot (Chapter IV), trees were able to afford a long period without irrigation and did not show significant decreases in plant physiological performance as well as in

the fruit growth over two years of study. This might be due to a possible "stress memory" but for sure the use of vigorous rootstocks characterized with deep root systems enhanced the plant resilience to water stress. In addition, deficit irrigation seems to improve fruit quality in terms of sugar content, but unfortunately the results shown in this study do not provide strong evidence of this relation. In conclusion, the irrigation scheduling in apricot is surely overestimated by the actual decision support system available in Emilia-Romagna region.

Deficit irrigation protocols should be developed to optimize water use, fruit production and quality, especially in terms of nutraceutical components.

Hereupon, more research is required to better understand how to further enhance crop performances under climate changing conditions. In fact, it is no longer sufficient to study only the effect of one type of stress (e.g. drought stress, salt stress, heat stress, etc.) on tree physiology but it is important to understand how the interactions of these abiotic stresses challenge plant physiological responses.

It should be considered, however, another important point. The water stress imposed on different fruit crops under two years of study each, found as a general conclusion that the decrease in the irrigation water did not show a straightforward decrease in plant physiological performance. This can be due to the miscalculation of the real water needs of the considered fruit crops. In these studies, irrigation was scheduled using the regional platform "Irriframe" (www.irriframe.it). This is a decision support system provided and used at national level where the advised water inputs are calculated on the basis of the soil water balance. However, the results shown here clearly demonstrate how soils are able to store and provide water to fruit crops. For this reason, there is the need to improve this important tool for an appropriate water irrigation management. Furthermore, there is also the need to study the behavior of fruit crops under more severe deficit irrigations. It is probable, in fact, that the application of lower water amounts will enhance the synthesis of specialized metabolites, with positive effects on fruit quality and human health. These hypotheses must be verified.