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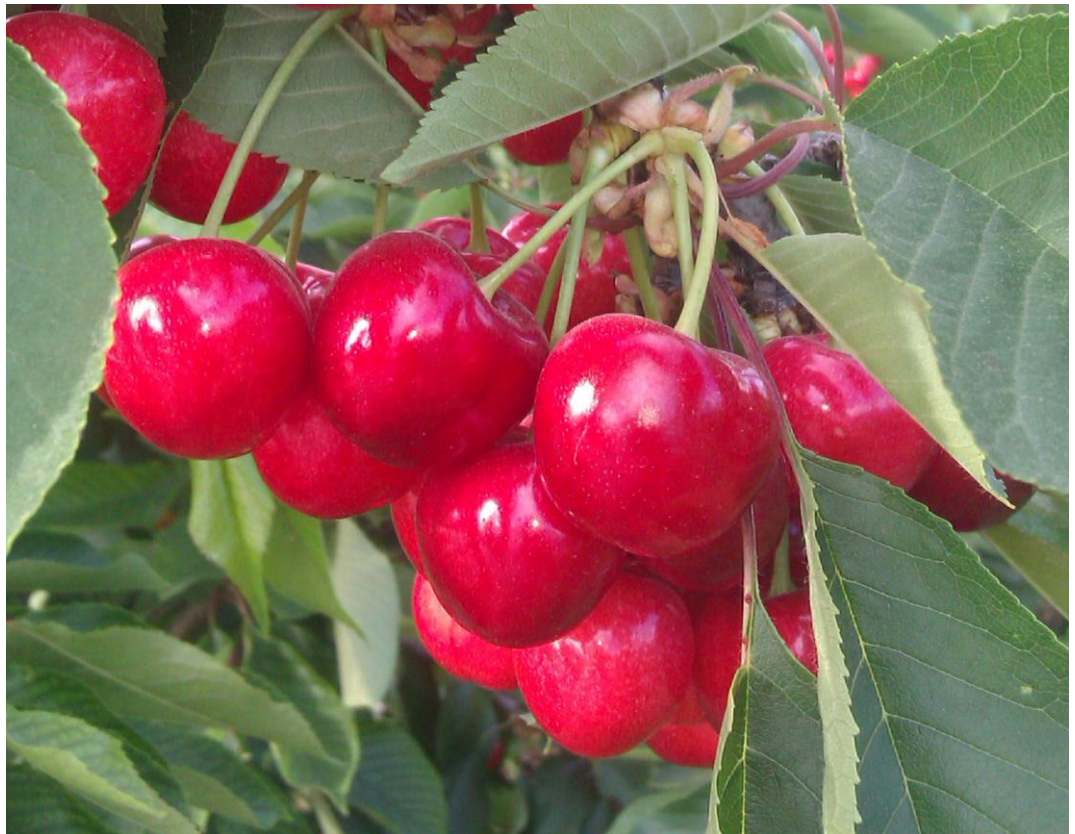


Universidad  
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*Agronomic and physiological basis for  
automating regulated deficit irrigation in  
sweet cherry trees*

*PhD Program in Advanced Techniques for  
Research and Development in Food and  
Agriculture*



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# **AGRONOMIC AND PHYSIOLOGICAL BASIS FOR AUTOMATING REGULATED DEFICIT IRRIGATION IN SWEET CHERRY TREES**

A Thesis submitted to

**Universidad Politécnica de Cartagena**

For the degree of

**Doctor of Philosophy**

In the International Doctoral School

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**Víctor Blanco Montoya**

La parte experimental de la tesis presentada se ha desarrollado en el marco de los proyectos *“Diseño de protocolos agronómicos y tecnológicos para el manejo del riego deficitario controlado en frutales a partir de redes inalámbricas de sensores”* (AGL2013-49047-C2-1-R) y *“Gestión automatizada del riego de precisión en frutales. Diseño de sensores y estudio de sensibilidad de indicadores de estrés hídrico”* (AGL2016-77282-C3-3-R), del Ministerio de Economía y Competitividad.

El trabajo de tesis doctoral que se presenta se acoge a la modalidad de tesis por compendio de publicaciones del Programa de Doctorado "Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario" de la Escuela Internacional de Doctorado de la Universidad Politécnica de Cartagena.

Consta de cuatro artículos, de los cuales dos de ellos han sido publicados en la revista "Agricultural Water Management" con un factor de impacto de 3,182 y otros dos en la revista "Scientia Horticulturae" con un factor de impacto de 1,76.

El nexo común de estos trabajos es el de promover el avance del conocimiento científico del funcionamiento hídrico del cerezo a partir del empleo de diferentes estrategias de riego y técnicas de cultivo. Para ello, se evaluaron las respuestas agronómica y fisiológica del cultivo y se estudiaron y compararon diferentes indicadores del estado hídrico del continuo suelo, planta, atmósfera con el fin último de aumentar la productividad del uso de agua.

Artículo I. **Blanco, V.**, Domingo, R., Pérez-Pastor, A., Blaya-Ros, P.J., Torres-Sánchez, R., 2018. *Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees*. Agricultural Water Management, 208:83-94. DOI: 10.1016/j.agwat.2018.05.021

Artículo II. **Blanco, V.**, Martínez-Hernández, G.B., Artés-Hernández, F., Blaya-Ros, P.J., Torres-Sánchez, R., Domingo R., 2019. *Water relations and quality changes throughout fruit development and shelf life of sweet cherry grown under regulated deficit irrigation*. Agricultural Water Management, 217:243-254. DOI: 10.1016/j.agwat.2019.02.028

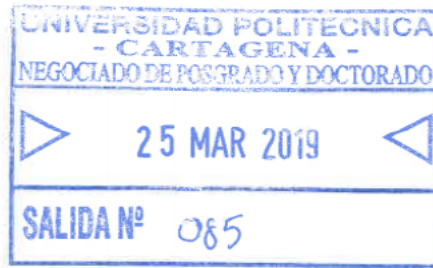
Artículo III. **Blanco, V.**, Torres-Sánchez, R., Blaya-Ros, P.J., Pérez-Pastor, A., Domingo, R., 2019. *Vegetative and reproductive response of 'Prime Giant' sweet cherry trees to regulated deficit irrigation*. Scientia Horticulturae, 249:478-489. DOI: 10.1016/j.scienta.2019.02.016

Artículo IV. **Blanco, V.**, Zoffoli, J.P., Ayala, M., 2019. *High tunnel cultivation of sweet cherry (Prunus avium L.): physiological and production variables*. Scientia Horticulturae, 251:108-117. DOI: 10.1016/j.scienta.2019.02.023





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**Sr. D. Víctor Blanco Montoya**

Visto el informe favorable del Director de Tesis y el Vº Bº de la Comisión Académica del Programa de Doctorado “Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario” para la presentación de la Tesis Doctoral titulada: **“Agronomic and physiological basis for automating regulated deficit irrigation in sweet cherry trees”** solicitada por D. VÍCTOR BLANCO MONTOYA, el Comité de Dirección de la Escuela Internacional de Doctorado de la Universidad Politécnica de Cartagena, en reunión celebrada el 25 de marzo de 2019, considerando lo dispuesto en el artículo 23 del Reglamento de Estudios Oficiales de Doctorado de la UPCT, aprobado en Consejo de Gobierno el 17 de diciembre de 2015,

**ACUERDA**

**Autorizar la presentación de la Tesis Doctoral a D. Víctor Blanco Montoya en la modalidad de “compendio de publicaciones”.**

Contra el presente acuerdo, que no agota la vía administrativa, podrá formular recurso de alzada ante el Sr. Rector-Magnífico de la Universidad Politécnica de Cartagena, en el plazo de un mes a partir de la notificación de la presente.

Cartagena, 25 de marzo de 2019

EL DIRECTOR DE LA ESCUELA  
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**CONFORMIDAD DE SOLICITUD DE AUTORIZACIÓN DE DEPÓSITO DE  
TESIS DOCTORAL POR EL DIRECTOR DE LA TESIS**

D. Rafael Domingo Miguel Director de la Tesis doctoral y D. Alejandro Pérez Pastor Codirector de la Tesis doctoral 'AGRONOMIC AND PHYSIOLOGICAL BASIS FOR AUTOMATING REGULATED DEFICIT IRRIGATION IN SWEET CHERRY TREES' / 'BASES AGRONÓMICAS Y FISIOLÓGICAS PARA LA AUTOMATIZACIÓN DEL RIEGO DEFICITARIO CONTROLADO EN CEREZO'

**INFORMAN:**

Que la referida Tesis Doctoral, ha sido realizada por D. VÍCTOR BLANCO MONTOYA, dentro del Programa de Doctorado Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario, dando nuestra conformidad para que sea presentada ante el Comité de Dirección de la Escuela Internacional de Doctorado para ser autorizado su depósito.

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

- Ciencias
- Ciencias Sociales y Jurídicas
- Ingeniería y Arquitectura

En Cartagena, a 11 de marzo de 2019

Rafael Domingo Miguel

EL DIRECTOR DE LA TESIS

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**COMITÉ DE DIRECCIÓN ESCUELA INTERNACIONAL DE DOCTORADO**





**CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL**  
**POR LA COMISIÓN ACADÉMICA DEL PROGRAMA**

D. Francisco Artés Hernández, Presidente de la Comisión Académica del Programa de Doctorado en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario.

**INFORMA:**

Que la Tesis Doctoral titulada, "AGRONOMIC AND PHYSIOLOGICAL BASIS FOR AUTOMATING REGULATED DEFICIT IRRIGATION IN SWEET CHERRY TREES", ha sido realizada, dentro del mencionado Programa de Doctorado, por D. Víctor Blanco Montoya, bajo la dirección y supervisión del Dr. Rafael Domingo Miguel y del Dr. Alejandro Pérez Pastor.

En reunión de la Comisión Académica, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización del Director de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por el Comité de Dirección de la Escuela Internacional de Doctorado.

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

- Ciencias
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- Ingeniería y Arquitectura

En Cartagena, a 21 de Mayo de 2019

EL PRESIDENTE DE LA COMISIÓN ACADÉMICA



*Francisco Artés Hernández*

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## LIST OF ABBREVIATIONS

- AGR: Absolute growth rate
- ANOVA: Analysis of variance
- BCSA: Branch cross sectional area
- BCSGR: Branch cross section relative growth rate
- BGR: Branch growth rate
- Ca: CO<sub>2</sub> concentration
- CI: Cracking index
- CIHEAM: International Center for Advanced Mediterranean Agronomic Studies
- CTL: Control treatment-full irrigation
- cv.: Cultivar
- CV: Coefficient of variation
- CWSI: Crop water stress index
- DAFB: Days after full bloom
- DOY: Day of year
- EC<sub>25°C</sub>: Electrical conductivity at 25°C
- ET: Evapotranspiration
- ET<sub>0</sub>: Crop reference evapotranspiration
- ET<sub>c</sub>: Crop evapotranspiration
- FAO: Food and Agriculture Organization of the United Nations
- FAOSTAT: Food and Agriculture Organization of the United Nations. Statistics division
- FDR: Frequency domain reflectometry
- FE: Fruit efficiency
- FRM: Farm treatment
- FTI: Number of fruit per trunk increment
- GR: Growth rate
- gs: Stomatal conductance
- IWUE: Intrinsic water use efficiency
- Kc: crop coefficient
- Kr: localization factor

- L\*: Lightness
- LA: Leaf area
- LA/F: Leaf area to fruit ratio
- LDVT: Linear variable differential transformer
- MDS: Maximum daily shrinkage
- MI: Maturity index
- N: North
- ns: No significance
- P: Significant level
- PFR: Photosynthetic photon flux rate
- Pn: Net photosynthesis
- PPFD: Photosynthetic photon flux density
- P-value: Significant level
- r: Correlation coefficient value
- RD: Regulated deficit irrigation treatments
- RDI: Regulated deficit irrigation
- RDM: Regulated deficit irrigation treatment which applied a mild water stress during preharvest and a medium stress during postharvest
- RDS: Regulated deficit irrigation treatment which applied a severe water stress during postharvest
- RH: Relative humidity
- $RH_{Max}$ : Maximum relative humidity
- $RH_{mean}$ : Mean relative humidity
- $RH_{min}$ : Minimum relative humidity
- S: Sensitivity according to Goldhamer and Fereres (2001)
- S\*: Sensitivity according to de la Rosa et al. (2014)
- SDI: Sustained deficit irrigation
- SI: Signal intensity
- $SI_{MDS}$ : Maximum daily branch shrinkage signal intensity
- SIAR: Servicio Integral de Asesoramiento al Regante

- SL64: Saint Lucie 64 (*Prunus mahaleb* L.)
- SSC: Soluble solids concentration
- TA: Titratable acidity
- TCSA: Trunk cross-sectional area
- TD: Trunk diameter
- Ta: Temperature of the air
- Tc: Temperature of the canopy
- Tleaf: Leaf temperature
- T<sub>Max</sub>: Maximum temperature
- T<sub>mean</sub>: Mean temperature
- T<sub>min</sub>: Minimum temperature
- tri: Irrigation treatment
- UNESCO: United Nations Educational, Scientific and Cultural Organization
- VPD: Vapour pressure deficit
- W: West
- WP: Water productivity
- WSI<sub>system</sub>: Water stress integral
- YE: Yield efficiency

## LIST OF SYMBOLS

- $\Psi_{nfruit}$  : Fruit osmotic water potential
- $\Psi_{pfruit}$  : Fruit turgor potential
- $\Psi_{fruit}$  : Fruit water potential
- $\Psi_m$  : Soil water matric potential
- $\Psi_{stem}$  : Midday stem water potential
- $\theta_v$  : Soil volumetric water content
- $\theta_{vFC}$  : Soil volumetric water content referenced to field capacity

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# CHAPTER 1

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## SCOPE AND OBJECTIVES

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The global sweet cherry production has sharply increased in the last decades. As consumer interest in this seasonal fruit keeps growing, growers from areas where cherries have not been traditionally cultivated exhibit interest, and consider its cultivation due to a demand exceeding the offer.

These new areas are interested in extending sweet cherry season and providing high quality fruit when there is a low supply on the market. To achieve these goals, it has been necessary to adopt new orchard systems, new cultivar/rootstock combinations adapted to different edaphoclimatic conditions, and drip irrigation systems.

Rain-induced fruit cracking is the major limiting factor for cherry production. However, regarding defect-free fruit, small size fruit is a big problem that can compromise cherry profitability and pose an obstacle in its exportation, and this is where irrigation plays a main role. Hence the importance of researching sweet cherry response to irrigation; particularly in trees grown in areas with great potential for their cultivation, but highly vulnerable to climate change and water scarcity such as the Region of Murcia (Spain) and the Region of Maule (Chile).

To cope with the current situation exposed above and with the purpose of overcoming future challenges, the main objective of this thesis dissertation is to generate and transfer technical and scientific knowledge which could help sweet cherry automatic irrigation scheduling in Mediterranean climate areas where water is a limiting factor. This requires the characterisation of sweet cherry tree water status, water relations, soil water availability and climatic demand, and the evaluation of its physiological and agronomical response. Thus, the overall aim was achieved by meeting the following secondary objectives:

- Identify which of the commonly used soil and plant water status indicators are more useful for irrigation scheduling of sweet cherry trees (Article I).
- Estimate plant water status from meteorological and soil water indicators that can be easily integrated in automated systems (Article I).
- Assess the effects of deficit irrigation and different environments on cherry growth, quality and cracking susceptibility at harvest and during postharvest life (Articles II and IV).

- Study the long-term effect (2015-2018) of different deficit irrigation strategies on adult sweet cherry trees water status and their vegetative and reproductive response in order to ascertain the most recommendable irrigation strategy in warm areas with scarce water resources (Articles II and III).
- Make progress in the investigation of the effects of crop protection strategies on sweet cherry vegetative and reproductive response and the applied irrigation water (Article IV).

# CHAPTER 2

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## STATE OF THE ART

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## 1. Sweet cherry cultivation

### 1.1. Origin and characteristics

Sweet cherry (*Prunus avium* L.) is the most important species from the subgenus *Cerasus*, which belongs to the genus *Prunus* of the Rosaceae family. This genus includes 400 species and among them, some examples such as almond, apricot, cherry, peach and plum represent some of the most important tree crops in temperate areas. The origin of sweet cherries is thought to be related to the Near East, the regions around the Black and Caspian Seas (Vavilov, 1951). Its cultivation was expanded throughout the Mediterranean Basin with the ancient Greek civilisation. In Spain, there are documents that report the presence of sweet cherry trees in the 14th century (Flores del Manzano, 1985).

Sweet cherry is a deciduous tree fruit crop which has shown high tree vigour and moderate to high chilling requirements (Samish, 1954). Sweet cherry trees are traditionally large (10 m) with an upright growth, although in commercial orchards trees are pruned and canopies are shaped (Spanish bush, Y-shaped hedges, spindle, etc.) in order to increase tree densities—which can vary from 667 to 1250 trees ha<sup>-1</sup>—and consequently precocity and productivity (Picture 1A and 1B). This fleshy non-climacteric fruit is highly appreciated by consumers because it is one of the first temperate stone fruits to ripen in spring-summer, due to its balanced flavour (sweet-acid), and its streaking appearance with a small size, round to heart shape and a vibrant red to mahogany colour (Crisosto et al., 2003). It is also appreciated by growers mainly because it is a highly valuable tree crop and, moreover, because of its short period of fruit development which leads to lower water requirements compared to other fruit trees, and because it potentially promotes to be managed with other tree crops in the same orchard (García-Montiel et al., 2010).



Picture 1. Traditional sweet cherry tree (A) and intensive sweet cherry orchard system (B).

## 1.2. Crop physiology

### 1.2.1. Tree growth cycle

Sweet cherry trees annual growth cycle is clearly influenced in temperate climates by temperature (Agustí, 2004). Thus, two periods can be distinguished according to tree physiology response to seasonal evolution: (i) one of dormancy (from late autumn until the spring of the following year with the budding) and (ii) one of vegetative and reproductive activity which approximately corresponds to an eight-month duration: spring, summer and early autumn (Table 1).

The dormancy is the annual period required in the deciduous species when tree activity is negligible. The latency stage is developed by the trees as a response to cold winter (Kaufmann and Blanke, 2017). It begins in autumn when daytime becomes shorter and sunlight intensity and crop exposition time to sunlight declines, as well as temperature. As a consequence, trees slow down their production of chlorophyll and begin a progressive accumulation of growing-inhibitor compounds, such as abscisic acid while the plant continues to absorb nutrients (Agustí, 2004). Eventually, leaves which after all season often end up damaged by weather conditions (sunburn), diseases or insects, shrivel. During the dormancy period, sweet cherry must accomplish its chilling requirements (Atkinson et al., 2013). Sweet cherry trends to lack of chilling, and chilling accumulations under 50 % of its necessities might produce erratic and long blooms which will penalise crop yield (Cortés and Gratacós, 2008).

Within the vegetative activity two periods, preharvest and postharvest, are easily distinguishable. However, another period, floral differentiation, which temporarily matches with late preharvest and early postharvest is also considered. Vegetative activity was thereby divided into three periods: (i) preharvest, which starts with the swollen buds and finishes with the harvest, and includes sprout, bloom, fruit complete development and most vegetative development, (ii) floral differentiation, which includes the first part of flower induction and first flower bud differentiation, 15-20 days after the first harvest and (iii) postharvest, the largest period within the vegetative cycle, which starts after harvest and flower differentiation.

Vegetative and reproductive activity division into three periods was undertaken in accordance with sweet cherry sensitivity to water stress (Table 1). Thus, preharvest is a short period in which bloom and fruit development matches sprout and principal leaf area development and current season shoot extension. This period is defined as a stage susceptible to water deficit (critical period) and due to its short duration water savings in this period might not be as important as in postharvest (Marsal et al., 2010). Floral differentiation was considered an individual period of vegetative activity. It has been reported in *Prunus* trees as a stage sensitive to water deficit (critical period) which could affect next season floral development. Yield decreases from slight in almond (Esparza et al., 2001) to severe in apricot (Brown, 1953) have been reported as a result of deficit irrigation during the floral differentiation of the previous year. In sweet cherry, flower differentiation has been described to overlap pre and postharvest (Engin and Ünal, 2007). It begins earlier than in other fruit crops, during the final part of the fruit ripening in preharvest (Koutinas et al., 2010). However, this can last for 20 to 56 days depending on the cultivar, rootstock or climate conditions, so consequently it finishes in postharvest (Guimond et al., 1998; Li et al., 2010; Watanabe, 1982). Postharvest is the period when there is no fruit in the tree. In sweet cherry during this period final primary vegetative growth and secondary vegetative growth take place. It is described as a suitable period to apply deficit irrigation in different prunus crops (Torrecillas et al., 2018), such as apricot (Laajimi et al., 2009; Pérez-Pastor et al., 2014), nectarine (de la Rosa et al., 2016; Thakur and Singh, 2013), peach (Gelly et al., 2004), plum (Intrigliolo and Castel, 2006) and sweet cherry (Blanco et al., 2018; Morandi et al., 2018).

Table 1. Sweet cherry annual calendar in the northern (NH) and southern (SH) hemispheres.

NH	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
SH	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
Periods	Dormancy			Vegetative and reproductive activity								Dormancy	
	Critical period						Non critical period						
	Preharvest						Postharvest						
	Bloom		Fruit development			Harvest	Floral Diff.						
			SI	SII	SIII								



### 1.2.2. Fruit growth cycle

Sweet cherry fruit follows a double sigmoid growth pattern which consists of three stages (Coombe, 1976). Stage I is the period of cell division, which supposes an exponential fruit growth. During stage II the growth rate drastically decreases until values are close to zero, the pit hardens and the embryo develops. Once the stone's endocarp lignifies and the embryo in the seed is completely developed, stage III begins. Stage III is characterised by cell enlargement and increase in growth rates, with, again, an exponential growth pattern. In this stage trees show a high demand of water and carbohydrates. Fruit size is, therefore, conditioned by the number of cells reached in cell division during stage I and their enlargement during stage III (Yamaguchi et al., 2004), and only stage II could be considered as a non-critical stage according to fruit growth.

Although the fruit development of sweet cherry always has got these three stages, the double sigmoid pattern is based on the growth of mid- and late ripening cultivars such as 'Bing', 'Sweetheart' and 'Regina' (Fig. 1A). In extra-early and early cultivars, such as 'Royal Dawn', 'Brooks' and 'Prime Giant' the stage II is shorter and is overlapped by stage I and stage III (Fig. 1B). In those cultivars, as fruit development is fast, it is not evident a sharply decrease of growth rates to values close to zero as it is noticeable in late cultivars.

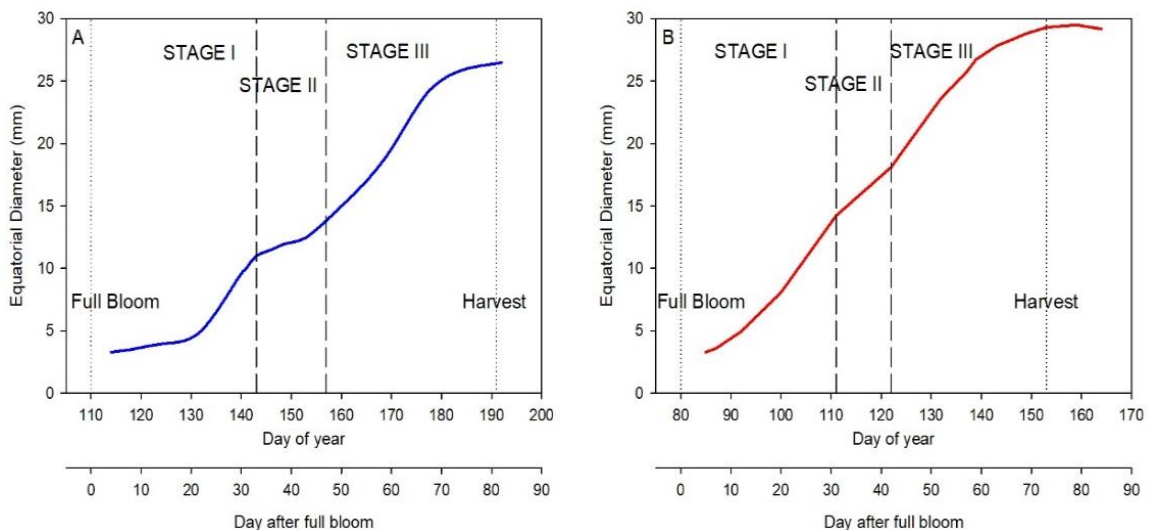


Fig. 1. Sweet cherry fruit growth pattern of a late cultivar 'Bing' (A) and an early cultivar 'Prime Giant' (B). (Source: (A) Zhang and Whiting, 2013; (B) prepared by the author).

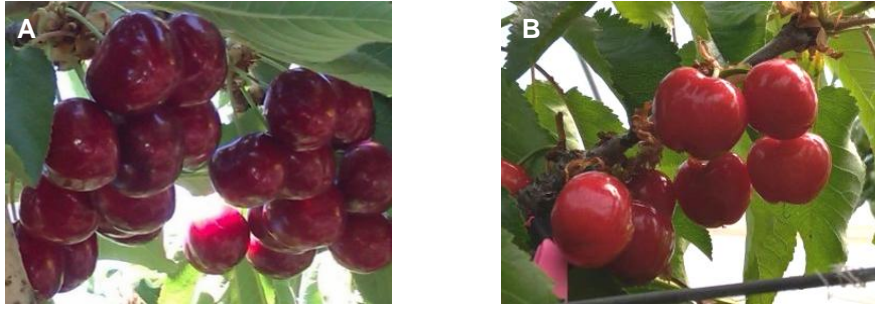
### 1.3. Cultivars and rootstocks

Of all existing possibilities among sweet cherry cultivar/rootstock combinations, this thesis dissertation studies the behaviour of two combinations: 'Prime Giant'/'SL64' and 'Royal Dawn'/'MaxMa14'. Both combinations, which were chosen to perform the trials shown below, are commonly used in commercial orchards.

#### 1.3.1. Cultivar

'Prime Giant' is an early sweet cherry cultivar with high productivity which was selected by Marvin Nies in California (USA) from hybrid parental ('Lady' x 'Ruby') and open pollination. Tree growth is semi-upright with medium to strong vigour. It blooms at the same time as 'Brooks' and 'Burlat' at early spring and ripens 7 days after them. It is self-sterile and its compatible pollinators are those cultivars from group II (S-alleles  $S_1S_3$ ), 'Brooks' and 'Lapins' are commonly used. Their fruits are large (equatorial diameter higher than 27 mm) with a shape similar to a spheroid oblate (sphere whose equatorial diameter is longer than the polar diameter), with a dark red-mahogany skin colour, a red pulp colour and high firmness (Picture 2A). Its flavour is intense with a balanced sweet acid ratio, presenting a soluble solids concentration of 18 °Brix. It is susceptible to cracking and double fruits and sensitive to bacterial canker. However, it is a cultivar recommended in warm regions with mild winters due to its low chilling requirements.

'Royal Dawn' is an extra-early sweet cherry cultivar obtained by Zaiger Genetics in California (USA) from the cultivar '32G500' x open pollination. It is self-sterile, and 'Tulare' and 'Lapins' are compatible pollinators. The tree has a vigorous growth, high upright branching and a good productivity; however, yield above 12 t ha<sup>-1</sup> delay the harvest. Blooming and ripening times are earlier than 'Prime Giant'. Their fruits are roundish of medium-large size (equatorial diameter between 26 and 30 mm), with a bright red skin colour, high firmness, good taste and a soluble solids concentration higher than 16 °Brix (Picture 2B). It is highly susceptible to stylar cracking and bacterial canker.



Picture 2. Details of 'Prime Giant' (A) and 'Royal Dawn' (B) sweet cherry fruit.

### 1.3.2. Rootstock

'SL64' is a *P. mahaleb* L. clonal rootstock selected by INRA (Picture 3A) in France. It is compatible with most sweet cherry cultivars and induces high productivity. 'SL64' is well adapted to calcareous and stony soil but is highly susceptible to root asphyxia, so it is only recommendable in well-drained soil. It has got high vigour and is low explorative. Cultivars grafted on 'SL64' rootstock grow about 20-30 % more vigorously than the same cultivars on 'MaxMa14'.

'MaxMa14' is a rootstock hybrid of *P. mahaleb* × *P. avium*, obtained by Brooks Nurseries (Picture 3B) in Oregon (USA) and well adapted to lime soil. It induces more precocity than 'SL64', moderate vigour and it is resistant to *Phytophthora cambivora* and *megasperma* and tolerant to bacterial canker. However, it is susceptible to severe droughts, but low susceptible to cherry leaf spot.



Picture 3. Details of 'SL64' (A) and 'MaxMa14' (B) rootstocks.

### 1.4. Advances in cultivation

Currently trends in sweet cherry cultivation are based on the combination of two factors, new conduction systems that promote higher tree densities (fruiting walls) and covers (nets, tents

and high tunnels) that protect the crop from adverse weather, rain and hail avoiding fruit cracking losses. High tunnels have emerged as the best cover method in highly valuable fruit crops such as sweet cherry (Picture 4). Apart from the fruit protection, the modification of the environment extends the harvest period, modifies the phenological response of the crop and increases the water use efficiency as soil evaporation decreases.

High tunnels protect fruit and bring forward the harvest, which significantly increases the fruit economic return. Their installation is justified only when fruit yield has a potential market willing to pay the added expense of this cultivation and its management costs (Lang, 2009).



Picture 4. Sweet cherry trees inside high tunnel.

### 1.5. Production

Sweet cherries are commercially produced in 68 countries, mainly in the temperate countries from the northern hemisphere, where Europe and Asia account for more than the 80 % of world production. Turkey is the largest producer according to FAOSTAT (2019) and during the period 2011-2017 the top six producing nations also included in the rank were United States of America, Islamic Republic of Iran, Italy, Spain and Chile (Fig. 2).

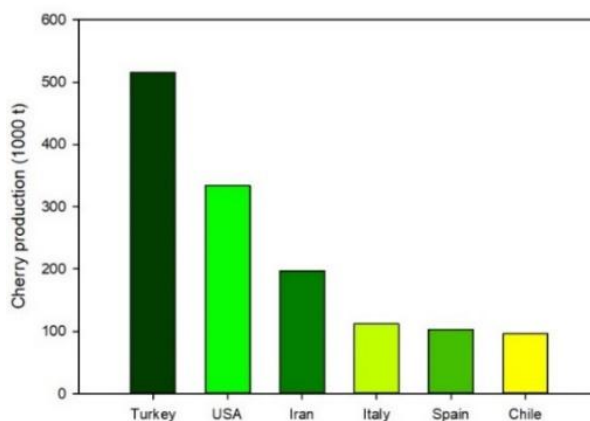


Fig. 2. Top 6 sweet cherry producer countries in the world for the 2011-2017 period. (Source: FAOSTAT, 2019).

World production and the harvested area have continued to increase until today since the late 1980s, early 1990s, when intensive orchard systems were imposed and new cultivar/rootstock combinations were developed to increase tree density, control tree size and extend picking season (Bujdosó and Hrotkó, 2017). From 2004 to 2017 sweet cherry world production and area increased 44 and 20 % respectively up to values of 2.4 million t and 416,000 ha (Fig. 3) with a matching increase in demand, particularly by China (Bravo, 2014), and production in countries such as Chile. Chile was a minor producer in 2001, but it increased its production by 363 % in 2016, reaching 23,000 ha and becoming the first country in volume and value of sweet cherry exports.

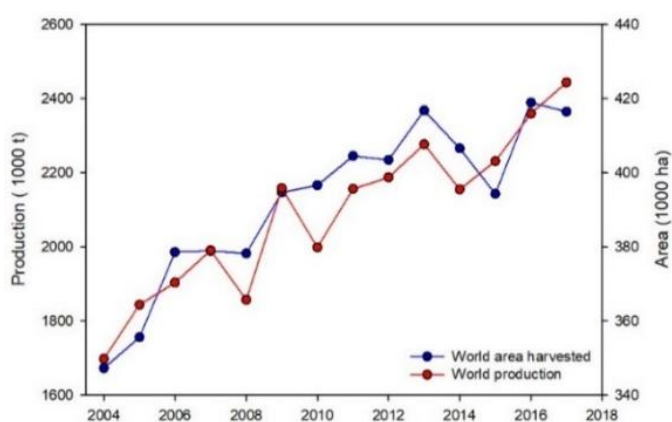


Fig. 3. Sweet cherry world area harvested and world yield for the 2004-2017 period. (Source: FAOSTAT, 2019).

In Spain in 2017, there were 27,600 ha that produced 114,400 t, which represented 6.6 and 4.7 % of the world's sweet cherry area and production (FAOSTAT, 2019). The production is mainly intended for domestic consumption or export to European markets. Sweet cherry tree is the third non-citric fruit tree with larger area in Spain after almond and peach trees. The most common sweet cherry cultivars are, for early season, 'Burlat', 'Prime Giant' and 'Frisco', for mid-season 'Santina', 'Summit' and 'New Star' and for late season 'Ambrunes', 'Sweetheart' and 'Lapins'; and the most common rootstock is 'SL64'. However, other rootstocks such as 'MaxMa14' or 'Marilan' are increasing their presence (Negueroles Pérez, 2005). Within Spain, Extremadura (Valle del Jerte) is the traditionally most important sweet-cherry-grower region, which in 2017 produced one third of the Spanish production (MAPA, 2018). Aragon (Valle del Ebro) was the second producer in Spain, 28 % of the total production. Both regions constitute more than 60 % of the Spanish sweet cherry production and are followed by Catalonia, Andalusia and the Valencian Community (Iglesias et al., 2016).

Although the Region of Murcia is not a traditional area to grow sweet cherry trees, its cultivation has got current and future possibilities due to the weather conditions there, which could promote early fruit development, move up the harvest time and get higher economic return to the grower (Guirao López, 2018). Thus, the area dedicated to this crop keeps on growing there; it has already increased from 184 ha producing 1,584 t year<sup>-1</sup> in 2014 to 332 ha producing 2,925 t year<sup>-1</sup> in 2017, all of them under irrigation, reaching the early cultivars (harvested in May–early June) prices between 4 and 3.6 € kg<sup>-1</sup> in 2016 and 2017 (CARM, 2017).

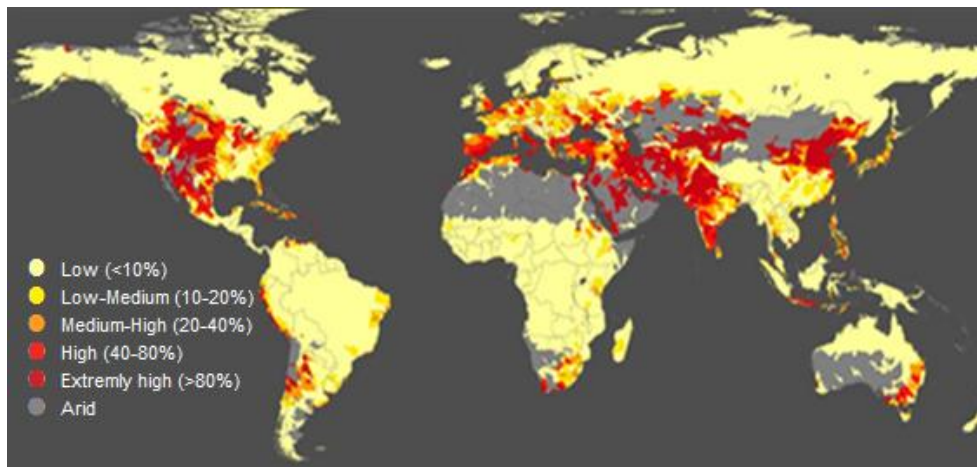
#### 1.6. Current perspectives and future challenges

Sweet cherry production could continue to increase in accordance with consumer demand, so its cultivation would expand to areas where it has not been previously grown, with more extreme weather conditions and lower water supply, as well as new conditions in areas where it is already, due to the climate change (Wenden and Mariadassou, 2017). This is the reason why field research will play a key factor in sweet cherry in the near future. Thus, new cultivars with lower chilling requirements, new rootstocks that promote narrow tree architectures which facilitate operations such as harvesting, studying tree response to covering technologies that protect the crop from hail and rain, improving irrigation management, optimising leaf area fruit ratio, etc. would achieve good yields of high quality fruit not just at harvest but during cold storage and will ensure crop profitability (Ayala and Lang, 2017).

#### 2. Climate change and agriculture

The areas under Mediterranean climate conditions such as the Mediterranean basin, California, Australia, South Africa and Chile, where agriculture is highly productive, are described as extremely vulnerable to climatic change (Giorgi and Lionello, 2008). These regions are already facing temperature increases, irregular rainfall patterns (storms) and severe water scarcity (Cosgrove and Loucks, 2015). Thus, there is an increasing concern between water resources and its efficient use (Picture 5). We are already in a water crisis and the agriculture as first water consumer is adapting to these conditions by improving water distribution channels and incorporating new technologies and sensors to crop management (Perry, 2018). However, it is

not enough to the future perspective, which foresees in 2050 that water stress in these areas would increase due to a water resources drop between 30 and 50 % (Milano et al., 2013).



Picture 5. Baseline water stress calculated as the ratio between water demand and water supply. (Source: WRI, 2013)

Agricultural research should focus not only on increasing crop yields, but also on increasing the productivity of limiting and irreplaceable factors such as water. The agricultural adaptation to this complex scenario is causing the need for further research in plant water relations within the soil–plant–atmosphere continuum in order to know and understand plant response to climate change. Thus, irrigation and crop management strategies which maintain yield and fruit quality without compromising food security and increasing water efficiency should be adopted (Fan et al., 2017).

### 3. Irrigation and water management

The advances in irrigation science and the application of adequate irrigation strategies and water management have involved (and involve) major improvements in agriculture's development. First, by increasing crop yield and consequently contributing to the development of rural areas, and now by helping the agricultural sector to adapt to new challenges such as the climate change, migrations and a rapidly growing world population with dwindling resources (Sauer et al., 2010). FAO (2018a) has highlighted how arid and semiarid regions that have kept traditional and intensive irrigation without implementing irrigation strategies to obtain a sustainable development have resulted in yield degradation, the collapse of the groundwater levels and finally the desertification of rural areas.

That is why irrigation scheduling should contemplate an efficient water use and must be based on scientific and technical knowledge. Irrigation has to be based on the crop water requirements according to its phenology and plant water status, but also on soil water availability and atmospheric demand. In order to increase irrigation water efficiency, irrigation should be, therefore, studied within the soil-plant-atmosphere continuum.

### 3.1. Soil-Plant-Atmosphere continuum

The soil-plant-atmosphere continuum (SPAC) analyses the flow of water throughout the plant which is driven by energy gradients from the soil to the atmosphere. This gradient is the result of the difference between water potentials, from high potential (less negative) in the soil, to a gradually lower potential in the root, stem and leaf, and finally to the lowest potential in the atmosphere.

With the objective of clarifying this pathway, van den Honert (1948) proposed a model similar to the Ohm's law. The model explains in steady state conditions that the water flux is proportional to the gradients of water potential and inversely proportional to the flow resistances. In the analogy, electrical current is replaced by water flow (transpiration rate,  $J_v$ ), voltage by water potential difference between two parts of the continuum ( $\Delta\Psi$ ) and electrical resistance by water resistance to the flow mass, osmosis and diffusion ( $R_n$ ), as shown in equations 1 and 2.

$$\text{Equation 1} \quad J_v = \frac{(\Psi_{\text{soil}} - \Psi_{\text{root}})}{R_1} = \frac{(\Psi_{\text{root}} - \Psi_{\text{stem}})}{R_2} = \frac{(\Psi_{\text{stem}} - \Psi_{\text{leaf}})}{R_3} = \frac{(\Psi_{\text{leaf}} - \Psi_{\text{air}})}{R_4}$$

$$\text{Equation 2} \quad J_v = \frac{\Delta\Psi}{\sum R} = \frac{(\Psi_{\text{soil}} - \Psi_{\text{air}})}{R_1 + R_2 + R_3 + R_4}$$

The SPAC considers the soil physics as soil water available to the plant (tension), the physiology of the plant as water transported throughout the plant from the roots to the stem and leaves to finally be transpired to the atmosphere (root, stem and leaf water potentials) and the atmospheric physics as water demanded by the atmosphere (vapour pressure deficit). Water energetic status ought to be measured, therefore, throughout the SPAC to validate irrigation strategies used, and according to the results obtained, crop water requirements can be assessed, and irrigation doses and scheduling can be improved, what consequently will avoid water losses and will improve irrigation water productivity.



### 3.2. Irrigation strategies

Water is indispensable for crop production and satisfying crop water requirements achieve yields close to maximum crop potential. When rainfall does not meet crop demand, irrigation water has to supplement it. As a result, water scarcity is the main factor limiting production in agriculture (Steduto et al., 2012). In this context, there is a widespread agreement on the fact that uncontrolled water stress affects crop production and development.

Sweet cherries are grown both under irrigation and rain-fed conditions. However, in warm areas with hot dry seasons vegetative growth, fruit yield and fruit size are negatively affected in trees under no irrigation (Proebsting et al., 1981). In Valle del Ebro in Navarra (Spain) where annual precipitation exceeded 750 mm, the uneven rain distribution causes that during summer the monthly accumulated precipitation was 35 mm; consequently, it was reported that rain-fed trees produced less than half of the fruit compared to trees under irrigation, 12.5 t ha<sup>-1</sup> (GEN, 1990).

However, in areas where water is scarce and its demand increases, irrigated agriculture must do an intelligent use of this resource, and sustainable management and utilisation of natural resources should be a main objective. Irrigation strategies such as regulated deficit irrigation (RDI) that increases water efficiency without decreasing crop yield, can be proposed in arid or semiarid regions susceptible to water deficit as an adaptation to water availability that improves water efficiency.

#### 3.2.1. Regulated Deficit Irrigation

RDI is an irrigation strategy based on reducing the amount of water supplied to the crop depending on its phenology and sensitivity to water stress. Thus, in periods when the crop is highly sensitive to water stress (critical periods), and water restrictions can affect its yield or/and quality, full irrigation is applied in order to ensure that plant water necessities are satisfied. Nevertheless, during the drought-tolerant phenological stages (non-critical periods) irrigation is limited. Consequently, the amount of water applied in RDI is lower than the amount calculated as optimal, so water productivity increases, vegetative growth decreases and yield or fruit quality is not negatively affected (Chalmers et al., 1981; Mitchell and Chalmers, 1982; Mitchell et al., 1986).

The effective implementation of this irrigation strategy requires a complete and deep knowledge of the crop physiology and the crop water requirements at each period of development in order to identify the crop critical phenological stages and foresee the effects of its application. RDI has achieved in numerous crops—especially fruit tree crops and vines—positive results. In horticultural and extensive crops, irrigation doses below crop necessities during their development are associated with decreases in yield, which consequently diminish crop profitability (Comas et al., 2019; Coyago-Cruz et al., 2019). Such yield reduction does not happen or are lower than those reported in vegetables and extensive crops in fruit trees under RDI due to the accurate period and intensity when deficit irrigation is applied. Thus, RDI is proposed as an irrigation strategy for fruit tree crops in regions sensitive to water scarcity, what might maximise grower's profit as well (García et al., 2004; Hargreaves et al., 1984). This reduces the investment in factors with high and rising prices, such as irrigation water and energy and, as it decreases excessive vegetative vigour, enhances tree aeration and solar interception, what diminishes fungal diseases, improves fruit development and also reduces agricultural costs associated to tree growth like pruning. Furthermore, not only does this strategy not affect tree yield, but it can positively affect fruit quality, increasing sweetness (soluble solids concentration), the colour (anthocyanins), etc,

However, RDI results vary depending on the crop, and in order to recommend RDI as an effective irrigation strategy in fruit trees, especially in drought-resistant species (or cultivars), the non-critical periods when water deficit is going to be applied must be stated, as well as the intensity of stress because the plant must be able to rapidly recover from the water stress once the non-critical period has finished (Pérez-López et al., 2018). It is necessary to know, therefore, which water stress indicator is the best option for each fruit tree crop and the threshold values that should not be exceeded.

### 3.2.2. Regulated deficit irrigation in sweet cherry

In sweet cherry, deficit irrigation is generally applied during postharvest, once the fruit has been harvested but the vegetative growth is still growing, which matches with summer, the season of maximum crop evapotranspiration. This is due to the sweet cherry growth pattern, where stage II overlaps stage I and III. Stage II is generally described as the stage when fruit growth stops

and the stone hardens—while vegetative growth keeps on growing. In sweet cherry, during preharvest vegetative and fruit growth occur simultaneously so, to avoid water stress during fruit growth, all preharvest is considered a critical period.

RDI experiments previously reported in sweet cherry have achieved water savings of 1,451 m<sup>3</sup> ha<sup>-1</sup>, 23 % of the water applied to the control treatment (6,279 m<sup>3</sup> ha<sup>-1</sup>) without reducing fruit yield (18-34 t ha<sup>-1</sup>) in The Dalles (Oregon, USA) for the combination 'Lapins'/'Mazzard' (Einhorn, 2012) and 1,617 m<sup>3</sup> ha<sup>-1</sup>, 33 % of the water applied to the full irrigated treatment (4,900 m<sup>3</sup> ha<sup>-1</sup>) with a fruit yield of 19 t ha<sup>-1</sup> in Torrente de Cinca (Aragon, Spain) with the combination 'New Star'/'SL64' (Marsal et al., 2009). Dehghanisani et al. (2007) applied sustained deficit irrigation in sweet cherry trees in the Moghan region (Iran) reaching water savings of 50 % and 75 % of the water applied to the control treatment (8,765 m<sup>3</sup> ha<sup>-1</sup>); however, both deficit treatments resulted in 10 to 30 % lower fruit yield than the control treatment.

As a main effect of deficit irrigation in sweet cherry trees different authors described a significant lower vegetative growth, measured as current season shoot extension (Dehghanisani et al., 2007; Podestá et al., 2011) and trunk cross sectional area (Nieto et al., 2017). Regarding fruit quality, Marsal et al. (2009; 2010) reported a higher soluble solids concentration in fruit from RDI trees in the combination 'Summit'/'SL64' but contrary values in the combination 'New Star'/'SL64'.

### 3.3. Water status indicators

RDI application requires, in addition to an accurate irrigation scheduling, high knowledge of the plant water status, which can be assessed by physiological and physical indicators (Table 2). Physiological indicators such as stem water potential, gas exchange, trunk diameter variations, sap flow and leaf temperature quantify plant water status directly or indirectly from the changes that it experiments. Physical indicators such as meteorological variables, soil water potential and soil water content measure changes in the environment that affect plant water status (Padilla-Díaz et al., 2018; Remorini and Massai, 2003).

Table 2. Classification and threshold values of common use soil and plant water status indicators.

	Water status		Threshold values		References
	indicator	Crop	Critical period	Non critical period	
Physiological indicators	Stem water potential	<i>P. avium</i> L.	-0.7 - -0.8 MPa	-1.5 MPa	Marsal et al., 2009
		'Summit'/'SL64'			
	Stomatal conductance	<i>P. avium</i> L.	150 - 200 mmol m <sup>2</sup> s <sup>-1</sup>	100 mmol m <sup>2</sup> s <sup>-1</sup>	Antunez-Barria, 2006
		'Bing'/'Mazzard' 'Skeena'/'Gisela6'			
	Net photosynthesis	<i>P. avium</i> L.	13 - 20 μmol m <sup>2</sup> s <sup>-1</sup>	10 - 15 μmol m <sup>2</sup> s <sup>-1</sup>	Gonçalves et al., 2005
		'Burlat'/'MaxMa14' 'Van'/'MaxMa14'			
	MDS	<i>P. avium</i> L.	200 μm d <sup>-1</sup>	350-450 μm d <sup>-1</sup>	Biel et al., 2012
	SI <sub>MDS</sub>	<i>P. persicae</i> Batsch.	1.1	1.4	de la Rosa et al., 2015
		'Flanoba'/'GF677'			
	BGR	<i>P. salicina</i> Lindl. Black Gold'/'Mariana'	25 - 30 μm d <sup>-1</sup>	5 - 10 μm d <sup>-1</sup>	Intrigliolo and Castel, 2006
TGR	<i>P. avium</i> L. 'Brooks'/'MaxMa14'	50 μm d <sup>-1</sup>	5 μm d <sup>-1</sup>	Livellara et al., 2011	
Sap flow	<i>P. avium</i> L.	0.4 L m <sup>-2</sup> leaf area d <sup>-1</sup>	0.2 L m <sup>-2</sup> leaf area d <sup>-1</sup>	Abdelfatah et al., 2013	
CWSI	<i>P. avium</i> L. 'Z900'/'Gisela5'	0.15	0.4 - 0.5	Köksal et al., 2010	
Physical indicators	Soil water content related to field capacity	<i>P. avium</i> L.	85 %	60 - 55 %	Neilsen et al., 2014
		'Cristalina'/'Gisela6' 'Skeena'/'Gisela6'			
Soil water potential	<i>P. dulcis</i> (Mill.)D.A.Webb 'Guara'/'GF677'	-30 kPa	-200 - -400 kPa	Puerto et al., 2013	

It is always better to count on multiple water stress indicators to manage irrigation scheduling. Thus, advantages and disadvantages of water indicators should be known in order to choose among them, those which adapt better to your specific circumstances. The election of the best water status indicators to sweet cherry irrigation management will be determined by the indicator's response, its sensitivity to rapidly detect and quantify water stress providing stable and representative measures with little variation. Moreover, other characteristics such as the

capability of being automated, providing continuous measures, low cost and low work associated with the obtaining of the measures should be considered.

### 3.3.1. Midday stem water potential

Midday stem water potential has been reported as the most straightforward water stress indicator of plant water status in fruit tree crops (Abrisqueta et al., 2015; Gonçalves et al., 2003; McCutchan and Shackel, 1992; Naor and Peres, 2001). Its measures are consistent and sensitive to irrigation regime as it integrates soil and environmental effects on whole tree water status. In addition, it is less dependent on weather conditions than leaf water potential (McCutchan and Shackel, 1992) and it has shown to be a reliable indicator of tree status even in the dormant period (Milliron et al., 2018). Its low variability, compared to other water status indicators, enables it to clearly distinguish between irrigation treatments despite other indicators are unable to differentiate between them (Abrisqueta et al., 2015; García-Orellana et al., 2013). Hence, it is proposed as a reference indicator (Naor and Peres, 2001).

In sweet cherry, Marsal et al. (2009) and Podestá et al. (2011) assessed midday stem water potential as a good tree water status indicator in irrigation management (Fig. 4). Neilsen et al. (2014) reported threshold values to identify detrimental stress in sweet cherry, although it has been reported that factors such as rootstock and cultivar combination affect significantly midday stem water potential (Gonçalves et al., 2003).

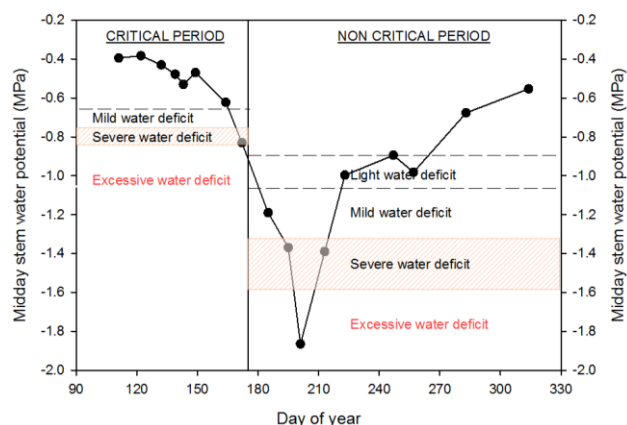


Fig. 4. Seasonal evolution of sweet cherry trees midday stem water potential irrigated according to grower's irrigation in a warm area under Mediterranean climate (Jumilla, R. Murcia, Spain).

On the other hand, midday stem water potential presents limitations; it is a one-off, labour-intensive measure that cannot be automated (Picture 6 A and B) (Esteves et al., 2015; Puerto et al., 2013).



Picture 6. Scholander pressure chamber (A) and covered leaf ready to be measured (B).

### 3.3.2. Gas exchange: Stomatal conductance and net photosynthesis

Stomatal conductance measures the degree of openness of leaf stomata. It is influenced among others by light intensity, vapour pressure deficit, temperature and relative humidity differentials between leaf and environment and water availability. In sweet cherry, as a consequence of water deficit, stomatal conductance decreases (Fig. 5). Stomatal aperture adjustment is an effective mechanism of the plant to deal with water deficit in order to regulate water flux throughout the plant and avoid water-loss dehydration. It is a consequence of leaf turgor loss and concentration increase of phytohormone abscisic acid (Blanco-Cipollone, 2017; Chater et al., 2014). Stomata reaction to plant water stress has been reported as a reliable indicator; however, it also shows high variability (Intrigliolo and Castel, 2006).

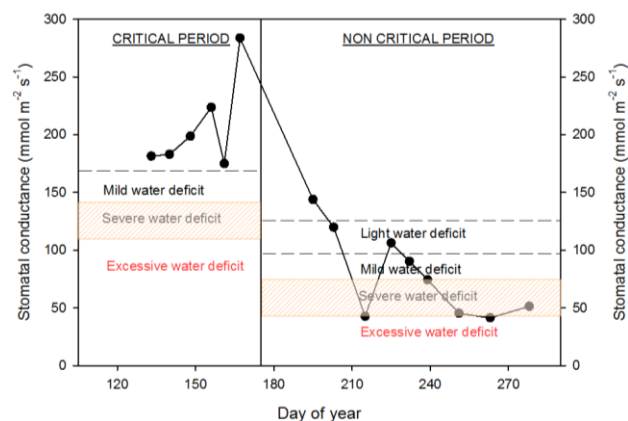


Fig. 5. Seasonal evolution of sweet cherry stomatal conductance irrigated according to grower's irrigation in a warm area under Mediterranean climate (Jumilla, R. Murcia, Spain).

Net photosynthesis is also affected by plant water stress. Stomatal closure is followed by a reduction of CO<sub>2</sub> availability to the chloroplast which triggers a decrease in net photosynthesis and slows plant growth and development (Antúnez-Barria, 2006). Concerning plant gas exchange analysis, its measurement is complex and it is not yet widespread amongst commercial orchards (Picture 7).



Picture 7. CIRAS-2 portable photosynthesis system.

### 3.3.3. Branch diameter variations

Branch diameter variation follows a circadian pattern. In the late afternoon, and mainly at night, as leaves' stomata close, plants can rehydrate their vascular tissues from the water available in the soil. Thus, their trunk and branch diameters grow during the night until dawn when they reach the maximum. Once the sun rises, stomata open, transpiration starts and plant water reserves in the vascular tissues decline as the vapour pressure deficit increases, consequently decreasing branch diameter, which reach its daily minimum in the early afternoon. From continuous measures of branch diameter, two indicators are obtained: maximum daily shrinkage (MDS) and branch growth rate (BGR). MDS is the resultant value of the difference between the maximum and the minimum daily branch diameter, and BGR is the difference between the maximum daily branch diameter of two consecutive days (Goldhamer and Fereres, 2001). Among the indicators obtained from diameter variations, MDS is the most used and the one that has showed better results in water stress detection in fruit trees and irrigation scheduling (de la Rosa et al., 2014; Ghrab et al., 2013; Intrigliolo and Castel, 2006; Ortuño et al., 2010; Puerto et al., 2013). In sweet cherry, MDS has been described as better water stress indicator than BGR (Livellara et al., 2011), and has been reported as a reliable water stress indicator (Fig. 6), particularly sensitive in early detection of water stress (Abdelfatah et al., 2013).

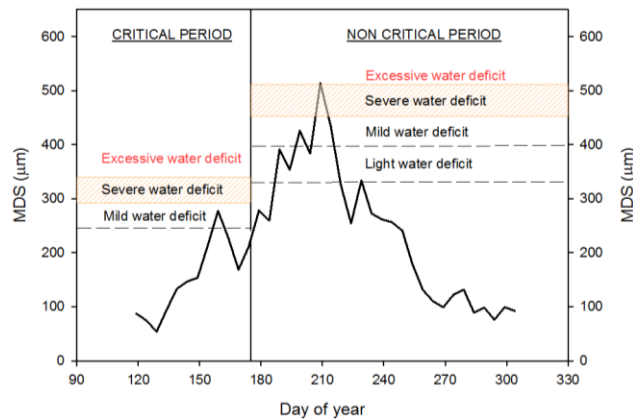


Fig. 6. Seasonal evolution of sweet cherry branch maximum daily shrinkage according to grower's irrigation in a warm area under Mediterranean climate (Jumilla, R. Murcia, Spain).

Furthermore, branch diameter variations can be automatable, which is very interesting in irrigation management (Picture 8). On the other hand, its measurement does not provide information by itself, since it needs to be compared to the measures obtained by a complete irrigated tree. MDS values are highly dependent of external parameters such as tree size, crop load, weather, etc. so consequently values obtained from research trials are difficult to interpret and extrapolate to commercial orchards in order to schedule irrigation. Goldhamer and Fereres (2001) proposed, therefore, instead of using MDS absolute values, using the signal intensity ( $SI_{MDS}$ ) which is calculated as shown in equation 3.

Equation 3

$$SI_{MDS} = \frac{MDS_{measured\ tree}}{MDS_{complete\ irrigated\ tree}}$$

This situation, despite the good results obtained in fruit crops, early water stress detection restricts its use in commercial orchards.



Picture 8. LVDT sensor installed in a tree branch measuring its diameter variations.

### 3.3.4. Sap flow

Sap flow is a sensitive water stress plant indicator that can be measured automatically. This indicator identifies the water availability in the plant in order to not restrict transpiration, as it



compares sap flow with the transpiration and estimates water use. It is reported as a sensitive plant water stress indicator by several authors in woody plants (Alarcón et al., 2000; Bhusal et al., 2019; Nicolás et al., 2005). The most common and successful method to measure sap flow in fruit trees is the compensation heat pulse method, which measures the velocity of a heat pulse between two points down- and up-stream by measuring the temperature in those points. The lower the velocity, the higher the plant water stress. This has been successfully used in sweet cherry to estimate tree water consumption (Juhász et al., 2013). In spite of the positive results achieved, its current implementation beyond research trials is limited.

### 3.3.5. Leaf temperature

Leaf (or canopy) temperature is an indirect indicator of plant water status that has been used for tree water stress detection. Water stress induces stomatal closure which consequently increases canopy temperature. Water release by plants over its surface (mainly leaf surface) is the temperature-controlling mechanism that crops have got for temperature regulation. The water evaporated by a vegetable surface regulates crop temperature depending on the environmental conditions (temperature, relative humidity, vapour pressure deficit, evapotranspirative demand). Crops whose water requirements are satisfied show lower canopy temperature than air temperature (ranging between 1 or 2°C below). However, when transpiration decreases canopy temperature increases and exceeds air temperature, generally values close to 2-4°C above air temperature, although differentials of 15°C have been recorded (Akkuzu et al., 2013).

In order to quantify crop water stress from this indicator, the crop water stress index was proposed (CWSI, Idso et al., 1978; Jackson et al., 1981):

Equation 4 
$$CWSI = \frac{[(T_c - T_a)_n - (T_c - T_a)_{wet}]}{[(T_c - T_a)_{dry} - (T_c - T_a)_{wet}]}$$

In equation 4,  $T_c$  is the temperature of the canopy and  $T_a$  the temperature of the air, in three different situations:  $n$  for the case under study,  $wet$  is the temperature differential when the crop is transpiring at the maximum potential rate under the same conditions of  $n$  and  $dry$  is the temperature differential when the crop is not transpiring. According to this situation, CWSI can

vary from 0 to 1. CWSI is 0 in non-water deficit conditions when stomata are completely open and the canopy is transpiring at its maximum potential rate, and CWSI is 1 when the plant is under the most extreme water deficit conditions and stomata are completely closed.

Considering that this indicator is automatable, the good results obtained in crop water stress detection and the latest advances of thermal imaging, its use in crop water status monitoring has drawn attention of researchers and producers and provides an interesting and promising stream of research (García-Tejero et al., 2018).

### 3.3.6. Meteorological variables

Traditional irrigation based on applying fixed amounts of water at specific time periods dramatically changed when irrigation scheduling incorporated into its calculation weather variables; mainly crop evapotranspiration (ET<sub>c</sub>), as it is defined as the amount of water both evaporated by the soil and transpired by the crop (Allen et al., 1998). And although the areas with remote and open access to climate networks and historical and real-time weather data are increasing, the cost of installation and maintenance of a complete weather station is so high that this implies a low density of stations and consequently a high number of growers that cannot access to representative climatic data (Collins, 2011). However, the advances in climate monitoring systems have developed low scale meteorological devices which are able to record and store specific weather data from growers' orchards, what improves irrigation management and water use (Lorite et al., 2015).

Among all weather parameters evapotranspiration (ET<sub>0</sub>) and vapour pressure deficit (VPD), which in turn integrate different climate variables, have shown to affect tree water status the most (Abrisqueta et al., 2015; Corell et al., 2016). In sweet cherry, a strong relationship has been reported between VPD and plant water stress indicators such as stem water potential (Blanco et al., 2018) and sap flow (Juhász et al., 2013), and between ET<sub>0</sub> and branch diameter fluctuations (Abdelfatah et al., 2013).

### 3.3.7. Soil water content

To know soil water content at root zone provides useful information of the amount of water present in the soil and available to the plant. It can be measured as the volumetric water content, the ratio of water in a specific volume of soil. As soil water content can be continuously measured, soil moisture sensors can be used to automate irrigation scheduling and activate irrigation when soil water content is below the desired values (Datta et al., 2017).

In order to use soil moisture sensors to manage irrigation, it is necessary to know soil water content at field capacity to adapt threshold values to each soil. Soil water content variations will determinate when and how much water to apply to the plant in each situation. The range of values where soil water content can be considered optimal highly depends on the soil texture and depth. Moreover, the results measured sometimes are difficult to replicate due to the high variability of the soil.

### 3.3.8. Soil water matric potential

Among all soil water deficit indicators, soil water matric potential has been described as the most useful indicator, since it contemplates not only soil water content but also soil properties and texture (number and size of pores) and surface properties and tension. Soil water matric potential is the potential derived from the necessary force exerted by the roots of the plants to extract water from the soil.

Monitoring soil water matric potential has been reported as a successfully method to improve irrigation scheduling (Shock and Wang, 2011). It is able to be automated and provides continuous measures of soil water availability to the plant (Fig. 7). This has made this technology to be already implemented in commercial orchards. Thus, advances in automated measures of soil water matric potential such as autocalibrated sensors, low cost and resistant devices would help to increase the measuring points and consequently diminish the high variability of the soil, which could suppose a great step.

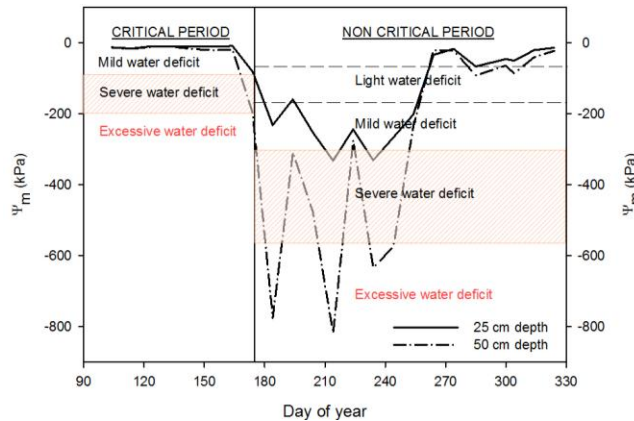


Fig. 7. Seasonal evolution of soil matric potential measured by MPS6 sensors (METER Group, Inc. USA) in a sweet cherry orchard according to grower's irrigation in a warm area under Mediterranean climate (Jumilla, R. Murcia, Spain).

### 3.3.9. Future perspectives

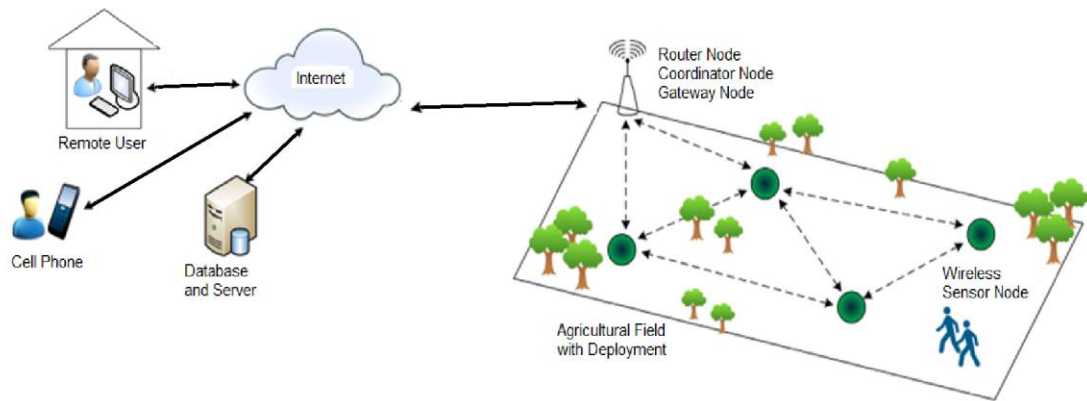
Nowadays, in commercial orchards meteorological and soil water status sensors are already a tool in irrigation management, while plant indicators such as sap flow or branch diameter fluctuations—although they also provide continuous measures—have not been as widely implemented. Stem water potential is considered the best plant water stress indicator, and in commercial orchards is commonly used; however, it is a one-off, destructive measure. Although there is already an automated sensor on the market capable of estimating stem water potential from the temperature gradient between tree sapwood surface and air “PSY1 Stem Psychrometers” (ICT International Pty Ltd, Armidale, Australia) and it has been successfully used in research trials (Wang et al., 2016), its commercial use is limited. As in the soil-plant-atmosphere continuum the plant hence responds to soil water availability and environmental conditions. To improve the use of those popular and continuous measuring sensors would entail a large step in knowing plant water status and would immediately help growers in the irrigation management decision-making.

## 4. Information and communication technologies in agriculture

FAO (2018b) projects that the global population will reach 9,700 million people in 2050, 2,400 million more than current population, making an increase of food production necessary. Resources are being more and more reduced and the increasing environmental concern demands agriculture to be more productive and sustainable, ensuring crop yields and environmental protection. This huge challenge requires global, efficient and smart solutions.

Information and communication technologies (ICTs) have led to a rapid change in all the principal development fields, and agriculture has not been left behind, adopting new technologies and changing progressively. Thus, precision irrigation incorporates ICTs (sensor networks, geographic information systems, satellite imaging, Internet of Things) to control crop, enhance the decision-making and optimise the use of water and inputs, pesticides, fertilisers, etc (López et al., 2015). Regarding the irrigation management, the amount of water and timing to apply it to the crop is based on knowing crop water requirements in accordance with the water status of soil, plant, and atmosphere, minimising water losses and avoiding soil salinity. ICTs such as remote sensing, wireless sensor networks, and mobile devices provide specific and real-time information to growers to maximise production efficiency, increasing water efficiency and decreasing carbon footprint and energy use (Bilali and Allahyari, 2018).

In this regard, and particularly in irrigation scheduling of RDI strategies which need a deep control of plant water status, soil water availability and atmospheric demand, the use of sensors and automatable water indicators are key factors to monitor and optimise crop and water management. Wired sensor networks are reliable and stable; however, compared to wireless sensor networks (WSN), wired networks show disadvantages in installation and maintenance, such as higher cost (mainly due to labour and cable costs) and location, sensor location distance is wired-limited (Ruiz-García et al., 2009). Moreover, WSN count on interesting characteristics, such as autonomy, low energy consumption and heterogeneity, and they are prepared to be connected to several sensors with different interfaces. Furthermore, its scalable architecture allows them to add new nodes to the network or easily change the configuration of the nodes. WSN are composed of several devices, commonly called nodes, which are connected wirelessly (Picture 9). In order to carry out a common objective, the different types of nodes can communicate with each other. The sensor nodes are the devices where the sensors are connected. Sensor nodes need router nodes to transmit the information to the coordinator node. The coordinator node collects the data and manages the network of sensor nodes. The coordinator node also acts as the gateway node, which is the node that sends data. The user from its computer or cell phone can connect to the gateway node and access the stored data (Bandur et al., 2019).



Picture 9. WSN connection scheme (Source: Navarro-Hellín, 2016).

WSN are prepared to work under hostile meteorological conditions and tolerate electronic and communication failures. Regarding its appearance, nodes are small and do not hinder farmer's work. Due to the problems of wired sensor networks and the attractive characteristics of WSN— although in research trials with high sensor density wired networks have provided good results— in commercial orchards the trend is to install WSN (Navarro-Hellín, 2016).

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# CHAPTER 3

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## ABSTRACTS OF THE SCIENTIFIC ARTICLES

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## ARTICLE I:

### Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees

#### Objective

The main objective of this article was to identify which of the commonly used soil and plant water status indicators is the most useful for deficit irrigation scheduling of 'Prime Giant' sweet cherry trees. Another purpose was to estimate midday stem water potential from meteorological and soil and plant water status variables that can be easily integrated in automated systems.

#### Materials and methods

The study was performed during the 2015 and 2016 growing seasons in a commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W). The trial was carried out on fifteen year-old mature 'Prime Giant' sweet cherry trees (*P. avium* L.) grafted on SL64 rootstock in a tree spacing of 5 m x 3 m. Three irrigation treatments were imposed: (i) a control treatment (CTL) irrigated at 110 % ET<sub>c</sub>; (ii) a regulated deficit irrigation treatment (RDI), irrigated at 100 % ET<sub>c</sub> during pre-harvest and flower differentiation and 55 % ET<sub>c</sub> during post-harvest; (iii) farmer treatment (FRM), irrigated according to the farmer's practices. Treatments were distributed according to a completely randomised block design with four replicates per treatment.

The plant water status was monitored approximately every ten days at noon by measuring midday stem water potential ( $\Psi_{\text{stem}}$ ) and stomatal conductance (gs). Continuous measurements of branch diameter fluctuations (BDF) were recorded by two dendrometers per replicate. From BDF, the maximum daily branch shrinkage (MDS) was calculated. Soil water status was measured from daily minimum soil volumetric water content ( $\theta_{\text{vFC}}$ ) and soil water matric potential ( $\Psi_{\text{m}}$ ). Daily agrometeorological parameters were provided by a weather station near the experimental orchard owned by SIAR. From these meteorological data vapour pressure deficit (VPD) was calculated. The sensitivity (S) of the studied water stress indicators was calculated according to Goldhamer and Fereres (2001). S is the result of the division of the Signal Intensity (SI) by the coefficient of variation (CV). Corrected sensitivity (S\*) proposed by De la Rosa et al. (2014) is calculated by the ratio between SI-1 and CV.

## Results and discussion

According to Girona et al. (2006), Naor and Peres (2001), Shackel et al. (2000),  $\Psi_{\text{stem}}$  can be considered as a reference water stress indicator in fruit trees. Consequently, water deficit indicators were ranked according to the goodness of fit of the calculated relations between these different indicators and  $\Psi_{\text{stem}}$ ,  $\text{MDS} = \theta_{\text{VFC}} > \Psi_{\text{m}} > \text{gs}$ . Moreover, MDS was the indicator that first detected water stress. However, the relationship between MDS and  $\Psi_{\text{stem}}$  achieved a maximum value of -1.3 MPa beyond which MDS and  $\Psi_{\text{stem}}$  were not linearly related. Thus, MDS as water stress indicator only has got a limited range in which it can be used to manage RDI. The evaluation analysis showed gs to be the plant indicator with the highest SI followed by MDS and  $\Psi_{\text{stem}}$ . Although  $\Psi_{\text{stem}}$  had a lower SI than gs and similar to that of MDS, S and S\* were much higher due to the low CV obtained. Soil water deficit indicators showed high SI values. However, they also had the highest CV.

The major drawback with  $\Psi_{\text{stem}}$  is that the measurement process cannot be automated and it provides one-off measurements. As the plant responds to soil water availability, as well as atmospheric demand,  $\Psi_{\text{m}}$  and VPD were related to estimate  $\Psi_{\text{stem}}$ . This relation describes two different situations to obtain an estimated  $\Psi_{\text{stem}}$  value, which depends on soil water availability and evaporative demand. If  $\Psi_{\text{m}}$  is lower than -30 kPa, there is a limiting soil water condition and the reference line is derived from  $\Psi_{\text{m}}$  and VPD, whereas if  $\Psi_{\text{m}}$  is higher than -30 kPa,  $\Psi_{\text{stem}}$  is mainly influenced by VPD.

If  $\Psi_{\text{m}} < -30 \text{ kPa}$   $\Psi_{\text{stem estimated}} = -0.3506 + 0.000642\Psi_{\text{m}} - 0.2143\text{VPD}$   $R^2=0.74$ ; p-value<0.01

If  $\Psi_{\text{m}} > -30 \text{ kPa}$   $\Psi_{\text{stem estimated}} = -0.1674\text{VPD} - 0,3197$   $R^2=0.78$ ; p-value<0.01

## Conclusion

The results obtained in the search for an overall indicator for use in irrigation management suggest the following order:  $\Psi_{\text{stem}} > \Psi_{\text{m}} > \text{MDS} > \text{gs} > \theta_{\text{VFC}}$ .  $\Psi_{\text{stem}}$  was seen to be the most reliable and stable water stress indicator as it clearly detected irrigation changes. Thus, we propose a  $\Psi_{\text{stem}}$  estimation model based on two easily available parameters, VPD and  $\Psi_{\text{m}}$ , which continuously register soil and atmosphere water status and obtain indirectly information regarding the plant water status.

## ARTICLE II:

### **Water relations and quality changes throughout fruit development and shelf life of sweet cherry grown under regulated deficit irrigation**

#### Objective

This article aimed at assessing the effects of deficit irrigation on plant and fruit water relations, fruit growth, yield and physicochemical characteristics at harvest and after cold storage and during subsequent retail conditions in 'Prime Giant' sweet cherries.

#### Materials and methods

The study was conducted in a 0.5 ha commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W) during two consecutive growing seasons, 2015-2016 and 2016-2017. The plant material consisted of fifteen year-old 'Prime Giant' sweet cherry trees (*Prunus avium* L.), grafted on SL64 rootstock, and spaced at 5 m × 3 m. Three irrigation treatments were applied: a control (CTL) irrigated at 110 % ET<sub>c</sub> and two regulated deficit irrigation treatments (RD): (i) RDM irrigated at 90 % of ET<sub>c</sub> during pre-harvest, 100 % of ET<sub>c</sub> during flower differentiation and 65 % of ET<sub>c</sub> during post-harvest; (ii) RDS, irrigated at 100 % of ET<sub>c</sub> during pre-harvest and flower differentiation and 55 % of ET<sub>c</sub> during post-harvest. Treatments were distributed according to a completely randomised block design with four replicates per treatment.

The plant water status was measured every seven-ten days by measuring midday stem water potential ( $\Psi_{\text{stem}}$ ) at noon. On the same days, fruit water potential ( $\Psi_{\text{fruit}}$ ) was measured. Fruit osmotic potential ( $\Psi_{\text{nfruit}}$ ) was measured in the same picked fruit as used to measure  $\Psi_{\text{fruit}}$ . Estimated fruit turgor potential ( $\Psi_{\text{pfruit}}$ ) was obtained as the difference between osmotic and fruit water potential according to Milad and Shackel (1992). Fruit size, equatorial and polar diameters (mm), fruit volume (cm<sup>3</sup>), fresh and dry unitary mass (g), fruit and pedicel colour, firmness (N), soluble solids concentration (SSC) and titratable acidity (TA) was measured every seven-ten days during fruit development at harvest, after 20 days of cold storage at 2°C and after 5 days (20 + 5) of shelf life simulation at 15°C.

## Results and discussion

$\Psi_{\text{fruit}}$  did not show significant differences among treatments and was strongly related to  $\Psi_{\text{stem}}$ . Moreover,  $\Psi_{\text{fruit}}$  was seen to be highly dependent on  $\Psi_{\text{nfruit}}$ .  $\Psi_{\text{fruit}}$  and  $\Psi_{\text{nfruit}}$  rapidly decreased as SSC rose after fruit's colour change, during the stage III of fruit development.  $\Psi_{\text{nfruit}}$  explained changes in  $\Psi_{\text{fruit}}$  better than  $\Psi_{\text{pfruit}}$ .  $\Psi_{\text{pfruit}}$  remained positive throughout fruit development. The fruit physical parameters and SSC evolution was characterised by a sigmoid growth pattern. Once fruit started to change colour, RDM led to higher SSC and redder colours than CTL and RDS, but at harvest trees of both deficit irrigation treatments bore darker cherries than CTL.

The year 2016 was a high cropping year and trees produced 43 % more kg per tree than in 2017 (42 vs. 29 kg fruit tree<sup>-1</sup>). As a result, fruit from 2016 was more prone to crack, 30 % smaller, 40 % firmer, less dark red, less sweet and less acid than the fruit from the same irrigation treatments in 2017. In neither year there were differences among irrigation treatments as regards yield and number of fruit per tree. However, RDM trees tended to produce fruit of smaller size than CTL, although without significant differences; in 2016 fruit from RDM was almost 1 g smaller than that from CTL. Regarding the quality parameters analysed (fruit and pedicel colour, firmness, SSC, AT), in 2016 there were no differences among irrigation treatments; however, in 2017 RDM fruit were sweeter and darker compared to the fruit from CTL. During 2016 storage trial, parameters such as size, SSC and TA remained stable throughout the experiment. On the other hand, fruit firmness increased significantly with time in cold storage and sharply declined during the shelf-life simulation. Fruit and pedicel colour decreased as time passed, particularly during shelf life, but only pedicel colour resulted affected by deficit irrigation. The pedicels from CTL fruit were significantly more brownish than those from RDS and RDM which remained green after storage.

## Conclusion

$\Psi_{\text{fruit}}$  was not as sensitive as  $\Psi_{\text{stem}}$  for identifying deficit irrigation. The application of RDM and RDS produced water savings of 36 and 40 % of the water applied to CTL treatment without significantly penalising fruit yield or quality. Fruits from RDS did not show any size reduction compared to CTL. Moreover, pedicel resulted in greener colour in RD fruit than in controls after 20 days at 2°C. When both RD treatments were compared, RDM did not improve RDS fruit quality.

### ARTICLE III:

#### **Vegetative and reproductive response of 'Prime Giant' sweet cherry trees to regulated deficit irrigation**

##### Objective

The objective of this article was to study the effects of different deficit irrigation strategies on the water status, yield and vegetative growth of adult 'Prime Giant' sweet cherry trees in order to optimise irrigation management in a semiarid area with scarce water resources.

##### Materials and methods

The experiment was conducted at a 0.5 ha commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W, altitude 670 m) from 2015 to 2018. The study was performed in fifteen year-old mature sweet cherry trees (*P. avium* L. 'Prime Giant') grafted on SL64 rootstock, at a plant density of 667 trees ha<sup>-1</sup>. The irrigation was applied during the dry period, from March before flowering until November. The experiment involved four irrigation treatments: (i) a control treatment (CTL) 110 % ETc; (ii) a sustained deficit irrigation treatment (SDI), irrigated at 85 % of ETc during pre- and post-harvest except for the floral differentiation when trees were irrigated at 100 % ETc; (iii) a regulated deficit irrigation treatment (RDI), irrigated at 100 % ETc during pre-harvest and flower differentiation and 55 % of ETc during post-harvest, and (iv) farmer treatment (FRM), irrigated according to the farmer's normal practice. Treatments were distributed according to a completely randomised block design with four replicates per treatment.

Measures of soil water matric potential ( $\Psi_m$ ) and soil water content ( $\theta_v$ ), midday stem water potential ( $\Psi_{stem}$ ), stomatal conductance (gs), net photosynthesis (Pn) and branch growth rates (BGR) were taken to evaluate soil and plant water status. At harvest, fruits from 5 central trees of each replicate were harvested and weighed. Similarly, fruits were counted in 5 kg samples to calculate the unitary mass and double and cracked fruit proportion in the sample. The number of fruit per tree was estimated. Vegetative growth was measured as pruning wood, canopy volume, shaded area, cumulate shoot growth and trunk cross-sectional area.

## Results and discussion

The reference crop evapotranspiration ( $ET_0$ ) showed a similar seasonal evolution all the years of the study, with an annual average sum of 1256 mm. Compared to CTL, RDI saved the greatest amount of water, 39 %, while SDI and FRM saved 28 % and 15 % respectively.

The seasonal trends in  $\Psi_m$  and  $\theta_v$  distinguished between the different irrigation strategies imposed in the three irrigation phases every year of the study.  $\Psi_{stem}$  was clearly influenced by evaporative demand and the applied deficit irrigation. RDI trees reached  $\Psi_{stem}$  values below -1.3 MPa all post-harvest; however, minimum value was measured in 2017 in FRM trees which resulted in  $\Psi_{stem}$  below -1.8 MPa. RDI trees resulted during post-harvest in  $g_s$  and  $P_n$  significantly lower than CTL trees,  $100 \text{ mmol m}^{-2} \text{ s}^{-1}$  and  $10 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  respectively. These reductions did not induce negative effects on next year yield and fruit quality; nevertheless, they affected vegetative growth, RDI trees in the last year of experiment resulted in the lowest canopy volume pruning wood and shaded area. BGR resulted in clear differences among treatments according to the irrigation treatment imposed which, at the end of each season, resulted in an accumulated BGR that was 1700  $\mu\text{m}$  lower in RDI trees than in CTL. The slight deficit irrigation applied in SDI trees during pre-harvest did not significantly decrease yield, but resulted in lower vegetative growth, especially in parameters, such as current season shoot growth. Moreover, it induced a slight higher number of fruits per tree and tended to lower size.

There was no significant effect of irrigation on yield any year. However, yield among years was significantly different; consequently fruit unitary mass was strongly influenced. Thus, a linear relationship was obtained [Unitary mass (g) =  $-0.1021 \text{ Yield (kg tree}^{-1}) + 13.67$ ]. The frequency of double fruit was not influenced by the irrigation treatment. On the contrary, cracking incidence was influenced by irrigation. Cherries of CTL were more prone to crack than those of RDI and SDI.

## Conclusion

A water saving of 39 % ( $2700 \text{ m}^3 \text{ ha}^{-1}$ ) compared to water applied to CTL in RDI trees did not penalise total fruit yield or quality, particularly fruit size. The regulated water deficit imposed during post-harvest in RDI trees decreased stomatal conductance and stem water potential, which resulted in vegetative growth lower than that obtained in CTL trees.



## ARTICLE IV:

### High tunnel cultivation of sweet cherry (*Prunus avium* L.): physiological and production variables

#### Objective

This article aimed at studying the effects of high tunnels environment in sweet cherry trees water status, yield, vegetative growth and fruit quality under a temperate Mediterranean-type climate.

#### Materials and methods

The trial was conducted in 2017 in a commercial orchard in the Central Valley of Chile (35° 1' S, 71° 32' W) with the early and highly-productive cultivar combination of 'Royal Dawn' on 'MaxMa 14'. Trees were trained as a Y-trellis, spaced at 4.5 m x 2.0 m. The experiment involved two treatments: (i) 'covered' trees under multi-bay high tunnels and (ii) 'open' trees under open field conditions 'control'. The treatments were distributed in a completely randomised block design with five blocks.

Temperature ( $T^a$ ), relative humidity (RH) and soil volumetric water content ( $\theta_v$ ) were recorded inside and outside tunnels. Tree water status was determined weekly by measuring midday stem water potential ( $\Psi_{\text{stem}}$ ) and stomatal conductance (gs). Current season shoot length, leaf number and fruit equatorial diameter per canopy layer at 0.8, 1.5, 2.0 m were measured weekly in marked fruits and shoots. Moreover, fruit growth was characterised each 7-10 days based on measurements of size, unitary mass and soluble solids concentration (SSC). Leaf area (LA) of individual spurs and extension shoots were measured on the same trees to estimate whole canopy LA and the ratio leaf area/fruit number (LA/F) according to Whiting and Lang (2004).

At harvest, yield of each canopy layer was recorded. In addition, 100 fruit per canopy layer per tree were collected for quality determination. Fruit showing visible cracking were recorded for each canopy layer in each tree in each treatment. Moreover, in order to assess fruit cracking potential, the cracking index was determined at laboratory. Fruit quality parameters included fruit size, mass, colour, SSC, titratable acidity (TA) and fruit firmness.

## Results and discussion

Compared to the open, the covers increased air temperatures by 5 to 10°C, slightly increased RH values and resulted in higher  $\theta_v$  due to the protected environment inside high tunnels. The higher temperatures inside the high tunnels during fruit development may have speeded cell division and cell expansion, since harvest was 8 days earlier in the covered trees. At harvest, open trees showed  $\Psi_{\text{stem}}$  values below -0.90 MPa, while  $\Psi_{\text{stem}}$  values of covered trees were -0.50 MPa, both treatments with stomatal conductance values above 200 mmol m<sup>-2</sup> s<sup>-1</sup>. There were no significant differences between the covered and open trees in LA/F ratio, where LA ranged between 186 and 200 cm<sup>2</sup> per fruit.

The total yield of covered and open trees was similar; although the fruit-size distribution in the open trees was concentrated between 26 and 28 mm, while that in the covered trees was between 28 and 32 mm. Regardless of fruit position in the canopy (layer - bottom, middle, top) the fruit from the covered trees was larger but had lower SSC (17 %) and firmness (7 %) than fruit from open trees.

13 days before harvest of the open trees (during Stage III) rain over two days (29 mm rainfall) caused 19 % cracking in the open trees. However, inside the high tunnel the incidence of cracking was only 3 %. Healthy fruit from the trees in the open were of higher cracking susceptibility than that from the covered trees. Fruit on covered trees showed lower cracking index than fruit from the open, suggesting likely better performance during storage.

## Conclusion

High tunnels stopped rain reaching the fruit surface and increased air temperatures compared to the open. Trees under high tunnels received 20 % lower irrigation water than open trees. Higher air temperatures under the covers speeded fruit growth and brought forward the dates of bloom and fruit development. As a direct consequence, covered trees were harvested 8 days earlier than trees in the open. There were no detectable differences either in yield, vegetative growth or in the LA/F ratio between covered and open trees. Fruit under the covers was 10 % larger but it had lower SSC and firmness.

# CHAPTER 4

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## GENERAL CONCLUSIONS

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The conclusions reported throughout the current PhD thesis dissertation describes the effects of different irrigation strategies (article I, II and III) and two different environments (article IV) on sweet cherry trees' reproductive and vegetative response and assess tree fruit growth, yield, vegetative growth, fruit quality, water savings, water productivity and some of the most common water deficit indicators used in irrigation management.

### **Water deficit indicators**

- MDS was the indicator that first detected water stress. However, it was seen that -1.3 MPa was the threshold value beyond which MDS loses its water deficit detection capacity. So MDS can only be used as a reliable water indicator in slight or mild deficit irrigation strategies.
- $\Psi_{\text{stem}}$  was the most reliable water deficit indicator, since, among the studied water deficit indicators,  $\Psi_{\text{stem}}$  was the one which showed the lowest coefficient of variation and high signal intensity and sensitivity.
- The integration of climatic demand (VPD) and soil water availability ( $\Psi_m$ ) in an equation allows estimating  $\Psi_{\text{stem}}$  under our trial conditions. The equation distinguished between two situations according to  $\Psi_m$ .

### **Effects of soil water deficit on agronomic and physiological responses**

- Tree's vegetative growth was more sensitive to soil water deficit than yield.
- Slight water deficits throughout the whole growing season resulted in lower vegetative growth without significantly reducing fruit yield. However, trees tended to produce slightly higher number of fruit per tree and fruit of smaller size.
- Trees submitted to postharvest severe water deficit did not result in lower tree yield or lower fruit unitary mass, but it affected tree water status decreasing  $g_s$  and  $\Psi_{\text{stem}}$ , which consequently reduced canopy volume, pruning wood and trunk cross sectional area increased.

### **Fruit quality**

- Trees submitted to deficit irrigation resulted in lower cracking losses, and were not affected by a higher occurrence of double fruit.
- In general, fruit of deficit irrigation treatments resulted neither in higher soluble solids concentration nor in intensive coloration than fruit of control trees, since only one out of four years of the study fruit from deficit trees enhanced SSC and colour.
- Deficit irrigation strategies tested did not penalise any quality parameter at harvest, or after 20 days of cold storage at 2°C and subsequent period of 5 days at 15°C.
- Fruit of trees submitted to deficit irrigation reduced its pedicel browning after 20 days at 2°C compared to fruit of control trees.

### **Water productivity**

- Trees under severe postharvest deficit irrigation resulted in the highest water savings, 39 % of the water applied to control trees, 2700 m<sup>3</sup> ha<sup>-1</sup> and season, and compared to trees irrigated according to the grower's experience, between 2200 and 1300 m<sup>3</sup> ha<sup>-1</sup> in 2015 and 2017 respectively.
- Regardless of the applied deficit irrigation strategy, all trees increased significantly water productivity and the ratio between fruit produced per annual trunk cross section increment compared to control trees without decreasing yield. However, only trees submitted to severe postharvest water deficit showed higher intrinsic water use efficiency and yield efficiency.

### **Environmental conditions**

- High tunnels environmental conditions moved sweet cherry harvest forward by more than a week respect to trees in the open; with the consequent water savings ( $\geq 20\%$ ), and improvement of the marketing conditions.
- Yield, vegetative growth and LA/F ratio did not result affected by protected cultivation; however, trees under high tunnels showed significantly lower cracking incidence and produced larger, less sweet, less firm and less susceptible to crack fruit than that of the trees in the open.

# ANNEX I

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## IMPACT FACTOR

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#### Article I

Blanco, V., Domingo, R., Pérez-Pastor, A., Blaya-Ros, P.J., Torres-Sánchez, R., 2018. Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees. *Agricultural Water Management*, 208:83-94. DOI: 10.1016/j.agwat.2018.05.021

Impact factor: 3.182 (Q1; 12/90 Water Resources)

#### Article II

Blanco, V., Martínez-Hernández, G.B., Artés-Hernández, F., Blaya-Ros, P.J., Torres-Sánchez, R., Domingo R., 2019. Water relations and quality changes throughout fruit development and shelf life of sweet cherry grown under regulated deficit irrigation. *Agricultural Water Management*, 217:243-254. DOI: 10.1016/j.agwat.2019.02.028

Impact factor: 3.182 (Q1; 10/87 Agronomy)

#### Article III

Blanco, V., Torres-Sánchez, R., Blaya-Ros, P.J., Pérez-Pastor, A., Domingo, R., 2019. Vegetative and reproductive response of 'Prime Giant' sweet cherry trees to regulated deficit irrigation. *Scientia Horticulturae*, 249:478-489. DOI: 10.1016/j.scienta.2019.02.016

Impact factor: 1.76 (Q1; 8/37 Horticulture)

#### Article IV

Blanco, V., Zoffoli, J.P., Ayala, M., 2019. High tunnel cultivation of sweet cherry (*Prunus avium* L.): physiological and production variables. *Scientia Horticulturae*, 251:108-117. DOI: 10.1016/j.scienta.2019.02.023

Impact factor: 1.76 (Q1; 8/37 Horticulture)

# ANNEX II

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## COMPILATION OF ARTICLES

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Article I

Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees.

Blanco, V., Domingo, R., Pérez-Pastor, A., Blaya-Ros, P.J., Torres-Sánchez, R., 2018.

Agricultural Water Management, 208:83-94.



## Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees

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### ARTICLE INFO

#### Keywords:

Maximum daily branch shrinkage  
Midday stem water potential  
Soil matric potential  
Soil volumetric water content  
Stomatal conductance

### ABSTRACT

A two-year experiment with sweet cherry (*P. avium* L. cv Prime Giant) trees was carried out to ascertain which of the following commonly used soil and plant water indicators is most effective for deficit irrigation scheduling:  $\Psi_{stem}$  (midday stem water potential), MDS (maximum daily branch shrinkage),  $g_s$  (stomatal conductance),  $\theta_v$  (soil volumetric water content),  $\Psi_m$  (soil matric potential). For this, soil and plant water relations, as well as the physiological and agricultural responses of trees to three different irrigation treatments, were evaluated. The irrigation treatments imposed were: i) a control treatment (CTL) irrigated at 110% of crop evapotranspiration ( $ET_c$ ) throughout the growing season, ii) a regulated deficit irrigation treatment (RDI), which met 100%  $ET_c$  at preharvest and during floral differentiation and 55%  $ET_c$  during the postharvest period and iii) a treatment based on normal farming practices (FRM).

MDS was the first indicator to detect water stress, while  $\Psi_m$  showed the highest sensitivity postharvest, when it was closely related with  $\Psi_{stem}$ . Consequently, a multiple linear regression equation based on average  $\Psi_m$  at a depth of 25 and 50 cm, and mean daily air vapor pressure deficit (VPD) was established to estimate  $\Psi_{stem}$ . The estimated  $\Psi_{stem}$  explained 84% of the variance in the measured  $\Psi_{stem}$ . Hence, the equation proposed can be used as a tool to estimate  $\Psi_{stem}$  and for irrigation scheduling. Based on the relation MDS vs.  $\Psi_{stem}$  and the observed agronomic response, a postharvest threshold value of 1.3 MPa is proposed for deficit irrigation management in 'Prime Giant' cherry trees.

### 1. Introduction

Because of the scarcity, water conservation in irrigated agriculture is of great importance in the Mediterranean Basin. Irrigation scheduling based on soil and plant water status sensors can contribute to increasing water productivity, thereby aiding water conservation. Besides, the use of sensors is crucial for precisely scheduling irrigation and to maximize water use efficiency in irrigated agriculture. For this, quantification of the spatial and temporal variability of the soil and plant water status is essential.

Regulated deficit irrigation (RDI) has been shown to increase both water productivity and growers' profits, especially in the case of fruit trees and vines (Behboudian and Mills, 2010; Fereres and Soriano, 2007; Torrecillas et al., 2018). Controlled water deficits are applied during phenological stages when trees are relatively tolerant of water stress (non critical periods) thus, minimizing or eliminating any adverse effects on yield and fruit (Mitchell et al., 1984). Implementing

validated RDI strategies in commercial fields can contribute significantly to water conservation. Having said that, most RDI experiments have been based on applying a percentage of crop evapotranspiration ( $ET_c$ ). However, replication of such RDI strategies, in commercial fruit tree orchards, even of the same variety, is limited by the difficulty of achieving a similar soil or plant water status. In part, this is due to the uncertainties associated with tree canopy architecture, the percentage of ground covered by the crop, the management of the soil surface and weeds and variability in soil type and depth (Ortuño et al., 2010). For this reason, soil plant sensing approaches, whereby the soil and plant water status threshold values are imposed experimentally, are preferable to ET based approaches.

Several soil and plant water indicators have been used to quantify tree water stress levels (Fernández and Cuevas, 2010; Ferreira, 2017; Jones, 2007). Among them, midday stem water potential ( $\Psi_{stem}$ ) is accepted worldwide as one of the most stable, reliable and accurate plant water status indicators for irrigation scheduling in many tree

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crops (Abrisqueta et al., 2015; Marsal et al., 2002; Naor and Peres, 2001; Shackel et al., 1997). Because of its high sensitivity to irrigation regime, Naor and Peres (2001) suggest that  $\Psi_{\text{stem}}$  can be used as a reference water stress indicator to compare the performance of other indicators. Nevertheless, measuring  $\Psi_{\text{stem}}$  is a destructive and labour intensive method and the equipment used to measure it cannot be integrated into an independent irrigation scheduling process, which limits its use (Puerto et al., 2013). Hence, the estimation of  $\Psi_{\text{stem}}$  from other easily automated and available variables, such as meteorological, soil and plant water variables might be considered of interest. Apart from midday stem water potential, other water stress indicators are often used in irrigated agriculture such as soil volumetric water content ( $\theta_v$ ) and soil matric potential ( $\Psi_m$ ) and within the field of irrigation research, leaf stomatal conductance ( $g_s$ ) and branch diameter fluctuations (MDS). The measurement of  $\theta_v$ ,  $\Psi_m$  and MDS is easily automated and several studies have demonstrated the influence of the first two on  $\Psi_{\text{stem}}$ , and a degree of correlation between  $\Psi_{\text{stem}}$  and MDS (Al Yahyai, 2012; Intrigliolo and Castel, 2007; Naor, 2004). Therefore, the use of these water stress indicators could contribute to the estimation of  $\Psi_{\text{stem}}$ .

Plant water stress indicators are thought to better integrate the environmental conditions, soil water content and evaporative demand to which the plant responds. However,  $\theta_v$  and  $\Psi_m$ , both soil water deficit indicators, are currently the most used in commercial plots as an aid to irrigation decision making (Navarro Hellín et al., 2015, 2016). This is in part due to the fact of their measurement is easily automated and can be subject to remote control. For it, any improvement in the estimation of  $\Psi_{\text{stem}}$  or even of any indicator concerning irrigation water management would be welcomed by growers in arid and semi arid areas. McCutchan and Shackel (1992) found a good correlation between  $g_s$  and  $\Psi_{\text{stem}}$  in prune trees. Leaf stomatal conductance represents water flow through the plant and the plant response to water use, although its measurement cannot be automated. Based on trunk or branch diameter fluctuations, the maximum daily shrinkage (MDS), which is the daily difference between maximum and minimum diameter values, can be calculated. MDS has been successfully used in irrigation scheduling in different fruit trees, including citrus (Ortuño et al., 2009), peach (Conejero et al., 2011), almond (Puerto et al., 2013) and nectarine (de la Rosa et al., 2013), and in vines producing table grape (Conesa et al., 2016). While this measurement can be automated, it needs signal intensity threshold values and reference equations, which are highly dependent on variety, tree size, crop load and other characteristics (Intrigliolo and Castel, 2007). Continuous measurements of volumetric content or soil water status have long been used for irrigation scheduling (Abrisqueta et al., 2012; Mounzer et al., 2008). A wide range of sensors based on capacitance and time domain transmission are available which can provide the continuous measurements of  $\theta_v$  and  $\Psi_m$  in real time. Moreover, they have the advantage of being easily coupled to data transmission systems.

Considering that in the soil plant atmosphere continuum the plant represents an intermediate system located in a water potential gradient between the soil and atmosphere, any measurement of plant water status will inevitably depend on the soil and air water status. It would therefore be interesting to have an equation that could be used to estimate the plant water status from soil and meteorological variables. This could be easily automated, while  $\Psi_{\text{stem}}$ , cannot.

As regards the crop in question, Spain is the second largest producer of cherries in Europe and the seventh largest producer in the world. Sweet cherry (*Prunus avium* L.) is an interesting stone fruit highly appreciated by consumers, and varieties such as Prime Giant are of particular interest due to its fruit quality attributes, large size, bright red colour, firmness, sweetness and balanced flavour (López Ortega et al., 2016; Nogueroles Pérez, 2005). *Prunus mahaleb* (SL64) rootstock has traditionally been used in sweet cherry trees in Spain since it is well adapted to the rocky and calcareous soils that predominate in the Mediterranean area. However, the roots of this rootstock have a low capacity to explore and root development is concentrated in the

irrigated area, particularly when the trees grow in arid or semiarid climates and have been provided with drip irrigation since planting (Bielorai, 1982; Paltineanu et al., 2016).

The main objective of this paper was to identify which of the commonly used soil and plant water status indicators is most useful for deficit irrigation scheduling of 'Prime Giant' sweet cherry trees. Another aim was to estimate midday stem water potential from meteorological and soil and plant water status variables that can be easily integrated in automated systems. To help achieve these objectives, the physiological response of 'Prime Giant' cherry trees subject to full and deficit irrigation is characterised over a two year period and  $\Psi_{\text{stem}}$  is represented with respect to different soil and plant water status indicators.

## 2. Materials and methods

### 2.1. Experimental site

The study was performed during the 2015 and 2016 growing seasons in a commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W). The experimental plot had an area of 0.5 ha. The trial was carried out on fifteen year old mature sweet cherry trees (*P. avium* L. cv Prime Giant) grafted on SL64 rootstock and 'Early Lory' and 'Brooks' as pollenizer. Tree spacing was 5 m × 3 m and average ground cover was around 55%. At the beginning of the experiment the trunk diameter of the vase shaped trees averaged 16.3 ± 0.18 cm. The soil is characterized by a sandy loam texture (15% clay, 17.5% silt and 67.5% sand), is moderately stony with a high organic matter content (6.3%) in the surface layer (5–35 cm depth), and an acceptable active limestone (2.7%), high assimilable phosphorus (108.67 mg kg<sup>-1</sup>) and adequate exchangeable potassium (0.32 meq 100 g<sup>-1</sup>) content. The irrigation water, which comes from a well, had an average electrical conductivity (EC<sub>25 °C</sub>) of 0.8 dS m<sup>-1</sup>, with maximum levels of sodium and chloride of 1.7 and 1.05 meq L<sup>-1</sup>, respectively. Horticultural practices (e.g. fertilization, weed control and pruning) were the same for all trees in trial and were carried out by the farm workers. Full bloom was in April, and annual pruning was carried out both years in late August (approximately 60 days after harvesting in June). The drip irrigation system consisted of a single drip line per tree row and three pressure compensated emitters per tree (4 L h<sup>-1</sup> discharge rate). The irrigation treatments were initiated each season in March before flowering at the beginning of the dry period and suspended at the end of November, the end of the dry period. Fertilization was applied in the drip irrigation water and followed the principle of re establishing the levels of nutrients taken up by mature trees; the fertilization program was the same for all the treatments and regardless of the water applied and consisted of 63, 30, 107 and 8 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and CaO respectively, applied in the drip irrigation water.

### 2.2. Irrigation treatments

Three irrigation treatments were imposed: (i) a control treatment (CTL) irrigated at 110% of crop evapotranspiration (ET<sub>c</sub>) to ensure non limiting soil water conditions; (ii) a regulated deficit irrigation treatment (RDI), irrigated at 100% of ET<sub>c</sub> during preharvest and the first days of flower differentiation (15–20 days after the first harvest) and 55% of ET<sub>c</sub> during postharvest (a non critical period), and (iii) farmer treatment (FRM), irrigated according to the farmer's normal practice. The average ET<sub>c</sub> satisfied by the FRM treatment during 2015–2016 periods was 98%, but fluctuated between 140 and 115% ET<sub>c</sub> in the preharvest period and between 90 and 50% ET<sub>c</sub> postharvest. Crop water requirements under drip irrigation (ET<sub>d</sub>) were calculated using the following equation: ET<sub>d</sub> = ET<sub>0</sub> × Kc × Kr, where ET<sub>0</sub> is the average reference evapotranspiration during the 3–5 days prior to applying the new irrigation scheduling, Kc is a crop specific coefficient, from March to November, whose monthly average values were: 0.30, 0.50, 0.90,

0.96, 0.96, 0.91, 0.69, 0.36 and 0.30, respectively (Marsal, 2012), and Kr is a localization factor (Feres et al., 1982) related to the percentage of ground covered by the crop ( $Kr = 0.90$ ).

Treatments were distributed according to a completely randomized block design with four replicates. Each replicate comprised a row of seven adjacent trees. Sensors were installed in the two central trees per replicate. The yield was measured from the five central trees, with the other trees serving as guard trees.

Irrigation frequency and timing varied during the season (March–November) from two irrigations per week early in the season to 4 irrigations a day during early mid summer, which was equivalent to 4 h per week and 7 h per day, respectively. In the last case and for the control treatment, irrigation was distributed according to the time scheduling: 01:00–02:00 h; 07:00–09:30 h; 13:00–15:00 h; 18:00–19:30 h. The irrigation at 7:00 h was common to and similar in all the treatments and was when fertirrigation was applied. The objective of high frequency irrigation applied during summer was to place the irrigation water where the root system was denser, keeping this area wetted and minimizing water losses through deep percolation.

### 2.3. Measurements

The plant water status was monitored approximately every ten days at 12:00–13:30 h (solar time) by measuring stem water potential at noon ( $\Psi_{stem}$ ) with a Scholander pressure chamber (Model 3000, Soil Moisture Equipment, Santa Barbara, CA), according to the methodology proposed by McCutchan and Shackel (1992) in 6 trees per treatment equipped with linear variable differential transformer (LVDT) sensors. The mature and healthy leaves selected were from the north quadrant close to the trunk, thus avoiding solar exposure. They were wrapped in small black polyethylene bags and covered with aluminum foil at least 2 h prior to the measurement. The water stress integral ( $WSI_{\Psi_{stem}}$ , MPa day) was calculated from the  $\Psi_{stem}$  values (May–November), as in Myers (1988) where the maximum  $\Psi_{stem}$  reached was  $-0.30$  MPa. Likewise, stomatal conductance ( $g_s$ ) was measured at solar midday in four sun exposed leaves per replicate from the outer canopy, with a photosynthetic photon flux density (PPFD)  $\approx 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , near constant ambient  $\text{CO}_2$  concentration ( $C_a \approx 380 \mu\text{mol mol}^{-1}$ ) and leaf temperature ( $T_{leaf} \approx 25^\circ\text{C}$ ) using a portable gas exchange system CIRAS 2 (PP Systems, Hitchin, Hertfordshire, UK).

Branch diameter fluctuations were recorded by two dendrometers (LVDT sensors, model DF  $\pm 2.5$  mm, accuracy  $\pm 10 \mu\text{m}$ , Solartron Metrology, Bognor Regis, UK) per replicate, each placed on a main tree branch away from direct sunlight. The sensors were installed on aluminum and invar holders to prevent thermal expansion. LVDT measurements were performed in differential input configurations. The maximum daily branch shrinkage (MDS) was calculated as the daily difference in diameter between the maximum and the minimum. MDS signal intensity ( $SI_{MDS}$ ) was calculated by taking the ratio  $MDS_{RDI}$  or  $FRM/MDS_{CTL}$ .  $SI_{MDS}$  is a dimensionless variable, where one is equivalent to absence of water stress and values above one indicate irrigation stress levels (Goldhamer and Feres, 2001).  $SI_{MDS}$  was calculated for the RDI treatment during 2016 in order to know which threshold level corresponded to the irrigation scheduling applied (55%  $ET_c$ ). Daily branch growth rate (BGR) was calculated as the difference in diameter between the maximum of two consecutive days (Goldhamer and Feres, 2001). From the measured branch diameters, the branch cross sectional area (BCSA) was calculated. Similarly, at the beginning, at harvest and at the end of the growing season, trunk diameter (TD) was measured with a tape measure (mod. “Pi meter” MF612 A). The measurements were always taken in the same place, a marked location about 0.20 m from the soil surface in the five central trees per replicate. Trunk cross sectional area (TCSA) was assumed as circle area and estimated from the trunk perimeter.

Soil volumetric water content,  $\theta_v$ , was obtained with three FDR sensors (Enviroscan, Sentek Pty. Ltd., Adelaide, Australia) per replicate

at 20, 40 and 70 cm depth located 0.23 m from the emitter and 1.5 m from the trunk, under the canopy projection. Based on these measurements and assuming a linear model between two consecutive depths, the soil volumetric water content at 25 and 50 cm depth was calculated. The daily minimum value of  $\theta_v$  is referenced to field capacity as a percentage of the maximum soil water content available in the soil,  $\theta_{vFC}$ . Likewise, the matric potential of soil water,  $\Psi_m$ , was measured in one tree per replicate by means of two thermal compensation capacitive sensors (MPS 6, Decagon Devices, Inc., Pullman, WA 99163 USA) at 25 and 50 cm depth and at a distance of 0.23 m from the emitter. The mean value of  $\Psi_m$  from 11:00 to 14:00 h (solar time) was calculated. Branch diameter fluctuations, volumetric water content and matric potential measurements were taken every 30 s and the datalogger was programmed to report means every 10 min. Two replicates per treatment were equipped using a wired platform (by one datalogger and two multiplexers Campbell Scientific, Logan, USA) and the other two replicates using a ZigBee wireless sensor network, WSN (Widhoc SS, Fuente Álamo, Spain) and configured in a star topology (Morais et al., 2008). Data access was by WIFI radio link provided by a local internet supplier. Daily agrometeorological records of air temperature, air relative humidity, rainfall as well as the crop reference evapotranspiration were provided by a weather station near the experimental orchard owned by SIAR (2018) (integral consulting service in agriculture). From the temperature and humidity data, the vapor pressure deficit (VPD) was calculated according to Allen et al. (1998).

Vegetative growth was measured as canopy volume and pruning wood. Canopy volume and tree shaded area was calculated annually according to Hutchison (1978) based on canopy height and diameters (across and within rows) before pruning. The pruning wood of each tree was individually weighed in the field. Pruning wood data are expressed as dry weight using a transformation formula calculated from aliquots of pruning fresh samples dried to constant weight in a ventilated oven (Dry Big, JP Selecta, Barcelona, Spain). Pruning wood dry weight (kg) = 0.5618 Pruning wood fresh weight (kg) + 0.0393.

At harvest (June 3rd and 10th in 2015 and 17th and 22nd in 2016), fruits from 5 central trees of each replicate were harvested and weighed. The individual yield of each tree was weighed to determine yield per tree. Similarly, at harvest, fruits were counted in 5 kg samples in order to calculate the unitary weight of the cherries. On 19th May 2015, a hail storm damaged the crop, and, to quantify the damage suffered, dropped fruit per tree were counted and weighed.

The sensitivity of the water stress indicators studied was calculated according to Goldhamer and Feres (2001) and the new approach proposed by de la Rosa et al. (2014). Traditional sensitivity (S) was calculated according to Goldhamer and Feres (2001). S is the result of the division of the Signal Intensity (SI) by the coefficient of variation (CV). Corrected sensitivity ( $S^*$ ) proposed by De la Rosa et al. (2014) is calculated by the ratio between  $SI - 1$  and CV.

Analysis of variance (ANOVA) was performed to determine the effect of the three different irrigation treatments on the soil and plant indicators, as well as to compare the estimation models for each irrigation season using the statistical software packages IBM SPSS Statistic 24 and Statgraphics centurion VI. Tukey’s test was used to detect differences between means with a significance level of  $P < 0.05$ . Linear and nonlinear relationships as well as regression analysis between variables were calculated with SigmaPlot 12.5 and Microsoft Excel (Microsoft Windows).

## 3. Results

### 3.1. Meteorological variables

The weather was classified as typically Mediterranean, with hot, dry summers and mild, wet winters. Both years had similar weather conditions, and the dry period was between March and November. Annual average temperature was  $16^\circ\text{C}$  with maximum temperatures of  $40^\circ\text{C}$ .

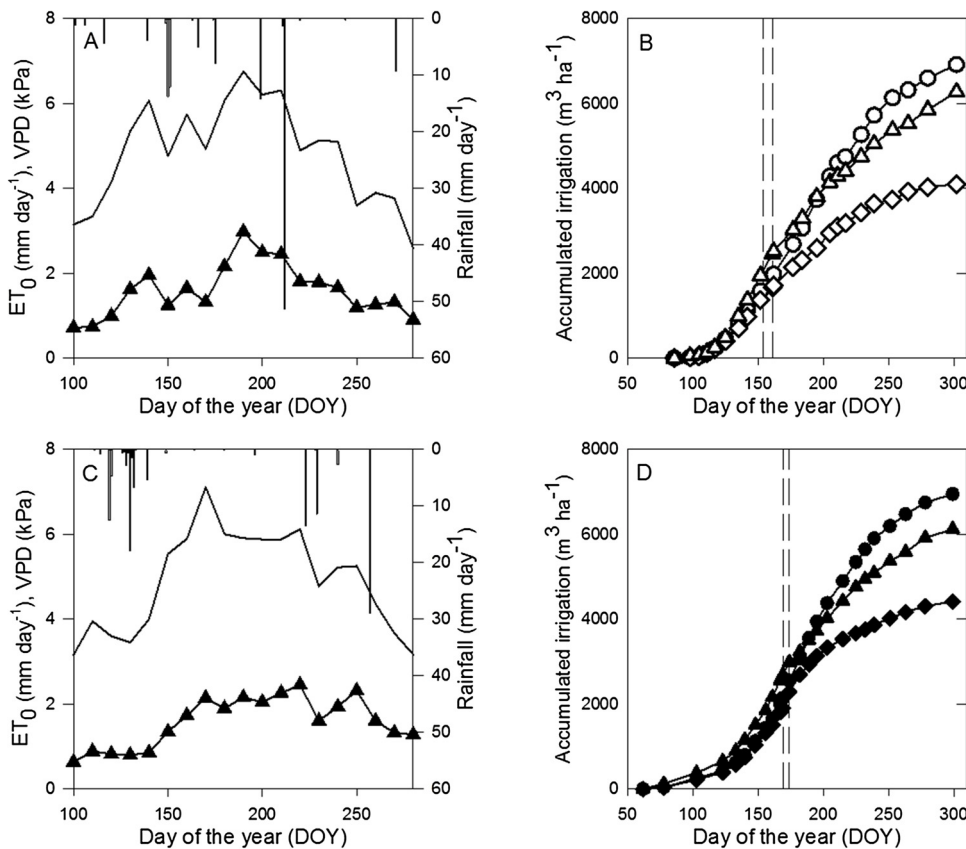


Fig. 1. (A and C) Meteorological variables: crop reference evapotranspiration ( $ET_0$ , continuous line), midday vapor pressure deficit (VPD, triangles) and daily rainfall (Rainfall, bars). (B and D) accumulated irrigation water applied to each treatment. Treatments: CTL, irrigated at 110%  $ET_c$  (circles); RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest (diamonds); FRM a treatment based on normal farming practices (triangles). A and B refer to 2015 and C and D refer to 2016. Vertical dashed lines indicate the fruit picks.

VPD reached daily mean values in summer of 4.0 and 3.8 kPa in 2015 and 2016, respectively. Annual rainfall was mainly distributed in spring and autumn, the 263 and 280 mm of rain that fell corresponding to 1272 and 1220 mm of reference crop evapotranspiration ( $ET_0$ ) in 2015 and 2016, respectively (Fig. 1A and C). This meant that the dry soil zone, or the area that received no water from the drippers (most of the inter row area) did not receive water during the period of greatest evaporative demand. This was also the period in which the water deficit was applied.

The average annual amount of irrigation water applied was 6981, 4156 and 6198  $m^3 ha^{-1}$  for CTL, RDI and FRM, respectively (Fig. 1B and D).

The meteorological variables measured mean temperature ( $T_{mean}$ ), maximum temperature ( $T_{Max}$ ), minimum temperature ( $T_{min}$ ), mean relative humidity ( $RH_{mean}$ ), maximum relative humidity ( $RH_{Max}$ ), minimum relative humidity ( $RH_{min}$ ), evapotranspiration ( $ET_0$ ) and vapor pressure deficit (VPD) were related to  $\Psi_{stem}$  (Table 1), only taking into account the days when there were non limiting soil water conditions ( $\Psi_m > -30$  kPa) in order to ensure the greatest relationship between  $\Psi_{stem}$  and the meteorological variables. Relative humidity vs.  $\Psi_{stem}$  showed an opposite relationship with air temperature, VPD and  $ET_0$  vs.  $\Psi_{stem}$ . VPD and maximum air temperature were the environmental parameters that best correlated with  $\Psi_{stem}$  ( $r > 0.8$ ), and,

as VPD is a more integrative variable, it was chosen to estimate  $\Psi_{stem}$ .

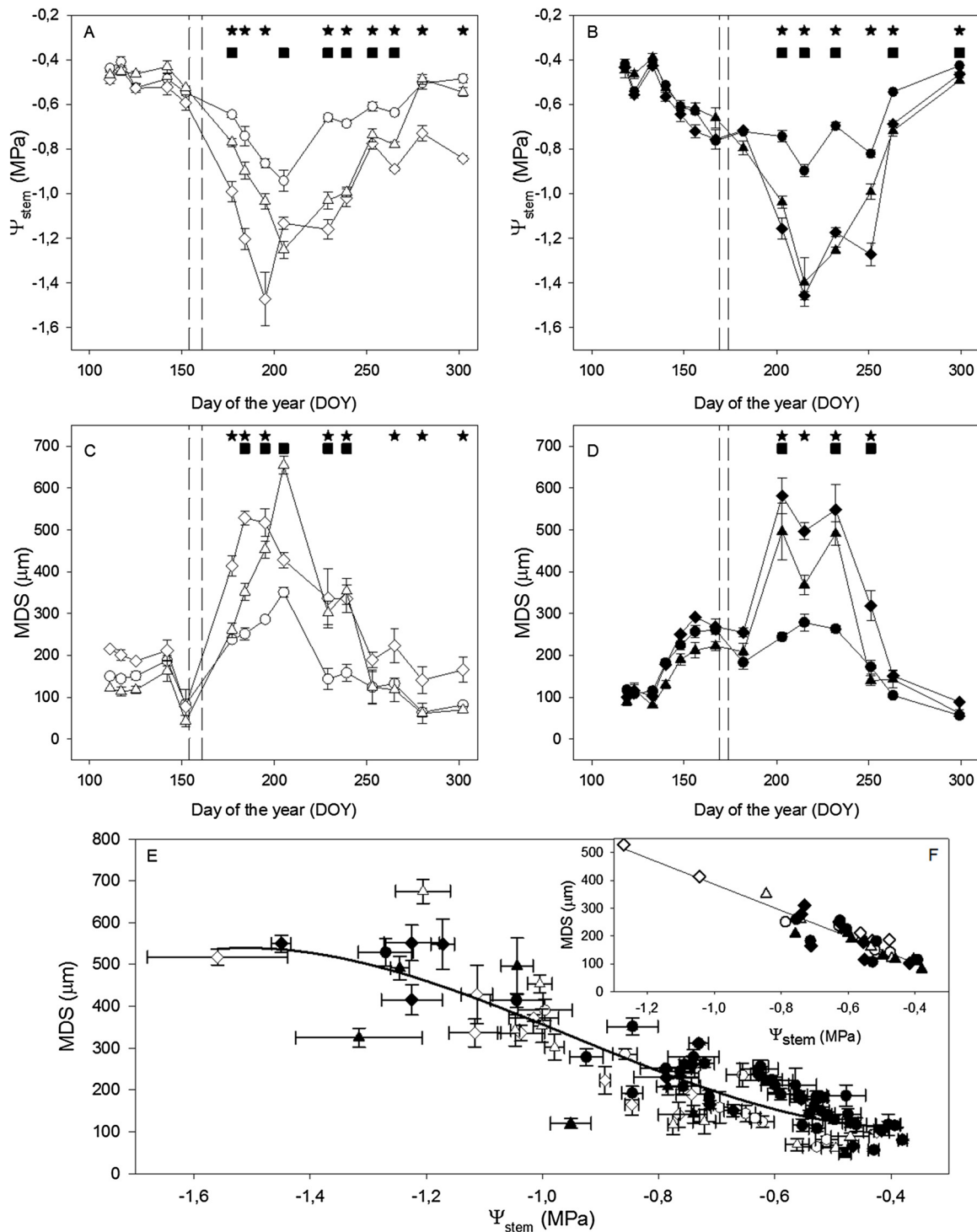
### 3.2. Midday stem water potential

$\Psi_{stem}$  showed similar behavior patterns both years of the study, with the highest mean values ( $\approx -0.4$  MPa) at the beginning and the end of the growing seasons (Fig. 2A and B), coinciding with the periods of lowest evaporative demand (Fig. 1). However, from late June onwards in both seasons, coinciding with the beginning of the water restriction,  $\Psi_{stem}$  values decreased as the evaporative demand rose, reflecting the progression of the water deficit period in FRM and RDI. The  $\Psi_{stem}$  values observed for the control trees were around  $-0.5$  MPa during preharvest and  $-0.7$  MPa during postharvest, values which indicate that the control trees were not water stressed (Fig. 2A and B). Before the deficit period, the preharvest period, hardly any differences in  $\Psi_{stem}$  were detected among treatments, the values remaining at a high level (around  $-0.5$  MPa), indicating a total absence of water stress. Mean while, differences between treatments were evident after harvest,  $\Psi_{stem}$  values reached a minimum of  $-0.92$ ,  $-1.45$  and  $-1.32$  MPa in 2015 and  $-1.00$ ,  $-1.56$  and  $-1.21$  MPa in 2016 in CTL, RDI and FRM respectively during midsummer. Stem water potential was closely associated with soil water content, which was particularly clear when the water supply was reduced. During postharvest,  $\Psi_{stem}$  values from the

Table 1  
Relationship of meteorological variables studied related to  $\Psi_{stem}$ . Correlation coefficient values (r).

	$T_{mean}$ °C	$T_{Max}$ °C	$T_{min}$ °C	$RH_{mean}$ %	$RH_{Max}$ %	$RH_{min}$ %	$ET_0$ mm	VPD kPa
$\Psi_{stem}$	0.78	0.82	0.58	0.57	0.27	0.59	0.68	0.85

Agrometeorological variables such as air temperature (mean temperature,  $T_{mean}$ , maximum temperature,  $T_{Max}$ , minimum temperature,  $T_{min}$ ) air relative humidity (mean relative humidity,  $RH_{mean}$ , maximum relative humidity,  $RH_{Max}$ , minimum relative humidity,  $RH_{min}$ ), evapotranspiration ( $ET_0$ ) and vapor pressure deficit (VPD) were related to midday stem water potential ( $\Psi_{stem}$ ).



**Fig. 2.** (A and B) Evolution of the midday stem water potential ( $\Psi_{stem}$ ). (C and D) MDS evolution the days  $\Psi_{stem}$  was measured. (E) Relationship between mean values of midday stem water potential and the maximum daily shrinkage for the data obtained for the whole season. (F) Relationship between mean values of midday stem water potential and the maximum daily shrinkage for the data obtained during late preharvest and early postharvest. Treatments: CTL, irrigated at 110%  $ET_c$  (circles); RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest (diamonds); FRM a treatment based on normal farming practices (triangles). A and C refer to 2015 and B and D refer to 2016. Each point is the mean value of 6 measurements. \* and ■ denote significant differences between CTL and RDI and between CTL and FRM, respectively, according to ANOVA ( $P < 0.05$ ).

RDI treatment fell 0.5 MPa in the first 20 days (Fig. 2A).

### 3.3. Maximum daily branch shrinkage

MDS varied from 30  $\mu m$  in all treatments to around 400  $\mu m$  in CTL, 600  $\mu m$  in RDI and 750  $\mu m$  in FRM, giving mean values of 197 and

180  $\mu m$ , 289 and 244  $\mu m$  and 258 and 216  $\mu m$  for CTL, RDI and FRM in 2015 and 2016 respectively. During 2015 and 2016 preharvest, mean MDS values were similar among treatments (Fig. 2C and D). However, in 2016 ten days after deficit irrigation was applied in RDI, differences in MDS increased, and the value in RDI trees was 33% higher than in CTL trees. When pre and postharvest data were considered together, a

polynomial relationship was found between  $\Psi_{stem}$  and MDS in all treatments, not only in 2015, but also in 2016 ( $R^2 = 0.78$ ). This third grade polynomial relationship showed that MDS increased as  $\Psi_{stem}$  dropped from  $-0.5$  MPa to a threshold value of around  $-1.3$  MPa (Fig. 2E). In our experiment, at values below  $-1.3$  MPa,  $\Psi_{stem}$  decreases were not directly related to higher MDS values. A close linear relationship was observed between these two variables during the last preharvest weeks (fruit growth period) and first postharvest days, from May to mid July, part of the period when deficit irrigation was being applied:  $DOY\ 125\ 205$  in 2015 and  $123\ 203$  in 2016,  $MDS\ (\mu m) = -472.69 \Psi_{stem}\ (MPa) + 86.21$ ,  $R^2 = 0.87$  (Fig. 2F).

The percentage of  $ET_c$  satisfied by irrigation in RDI during pre harvest, early postharvest, when flower differentiation takes place, and postharvest reflected  $SI_{MDS}$  mode values of 1.1, 1.0 and 1.6, approximately.

### 3.4. Gas exchange. Stomatal conductance

Two well differentiated phases could be distinguished in the seasonal evolution of stomatal conductance ( $g_s$ ) in both years of the study. We consider that during preharvest all the treatments showed similar behavior. However, during postharvest, RDI and FRM showed a different response to the water applied, both giving significantly lower values than CTL for all the measurements in 2016 and almost all in 2015 (Fig. 3A and B).

Two linear relationships were obtained between  $g_s$  and  $\Psi_{stem}$ : i) for the whole vegetative cycle, expressed both variables as the difference

between RDI and FRM (tri) respect to CTL (Fig. 3C) ( $\Psi_{stem\ tri} - \Psi_{stem\ CTL} = 0.0025 (g_{s\ tri} - g_{s\ CTL}) + 0.0326$  ( $R^2 = 0.68$ ), and ii) when only the summer data were taken into account, expressed as  $\Psi_{stem}$  vs.  $g_s$  (Fig. 3D)  $\Psi_{stem} = 0.0026 g_s - 1.4761$  ( $R^2 = 0.80$ ). This last linear relationship could also be interesting because this period corresponds to July and August, the time when the water deficit was being applied, accompanied by the greatest crop evapotranspiration.

### 3.5. Soil volumetric water content

During the preharvest period, the mean soil volumetric water content (expressed as a percentage of field capacity,  $\theta_{vFC}$ ) at 20 and 40 cm depth varied between 93 and 84% for the three treatments and both years. These  $\theta_v$  percentages correspond to non limiting soil water conditions and agree with the  $\Psi_{stem}$  values of the non water stressed plants (Fig. 2A and B). However, during postharvest, the  $\theta_{vFC}$  mean values in RDI and FRM fell to 60% in the first 10 days of deficit irrigation and continued falling, while the CTL values were higher than the 70% throughout the season (data not shown).

At 70 cm depth during 2015 and 2016, preharvest  $\theta_{vFC}$  mean values remained at about 75–80% reflecting the absence of a soil water deficit, with no significant differences among treatments; however, during postharvest this trend only continued in CTL, while in FRM and RDI the  $\theta_{vFC}$  dropped with the application of deficit irrigation and tended to remain stable except when it rained (Fig. 4A and B). The variations observed at 70 cm provided information about the fate of the irrigation water and the presence (or not) of drainage.

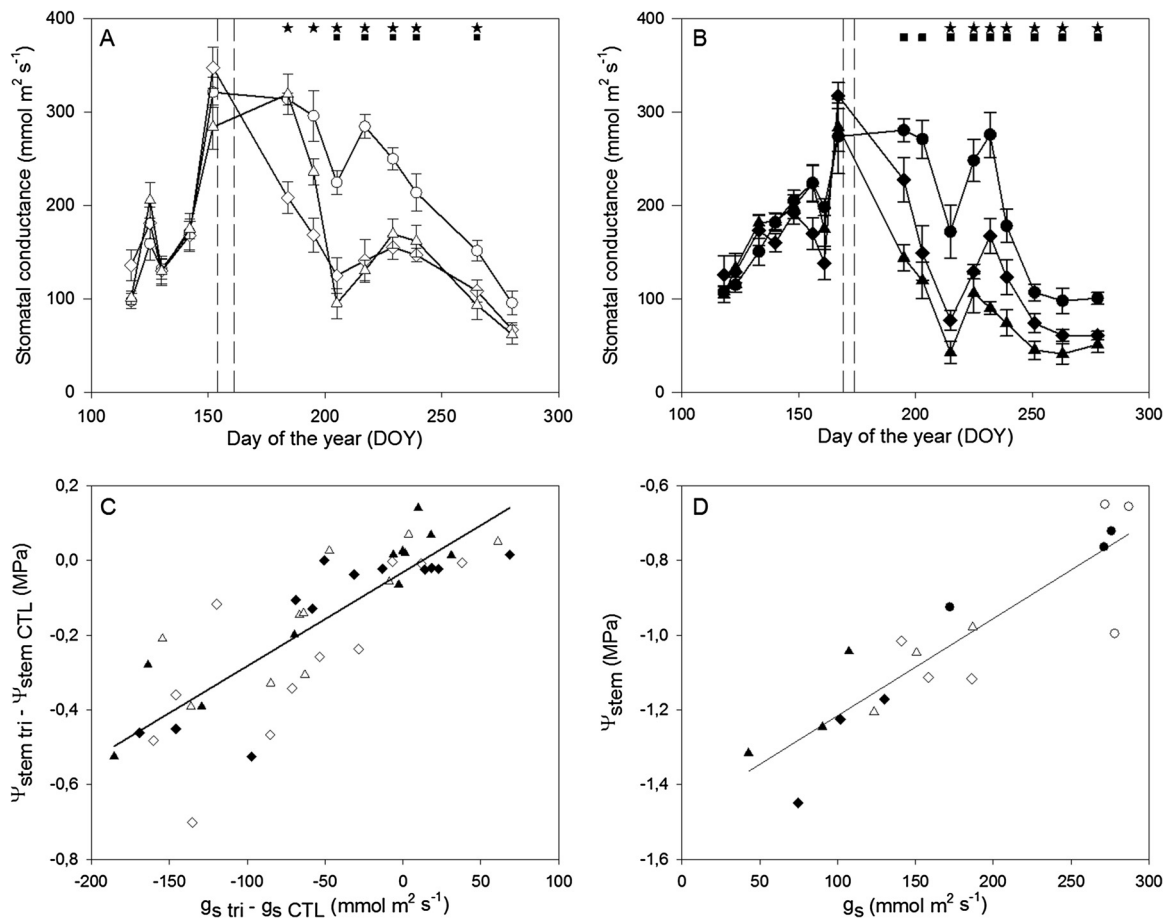
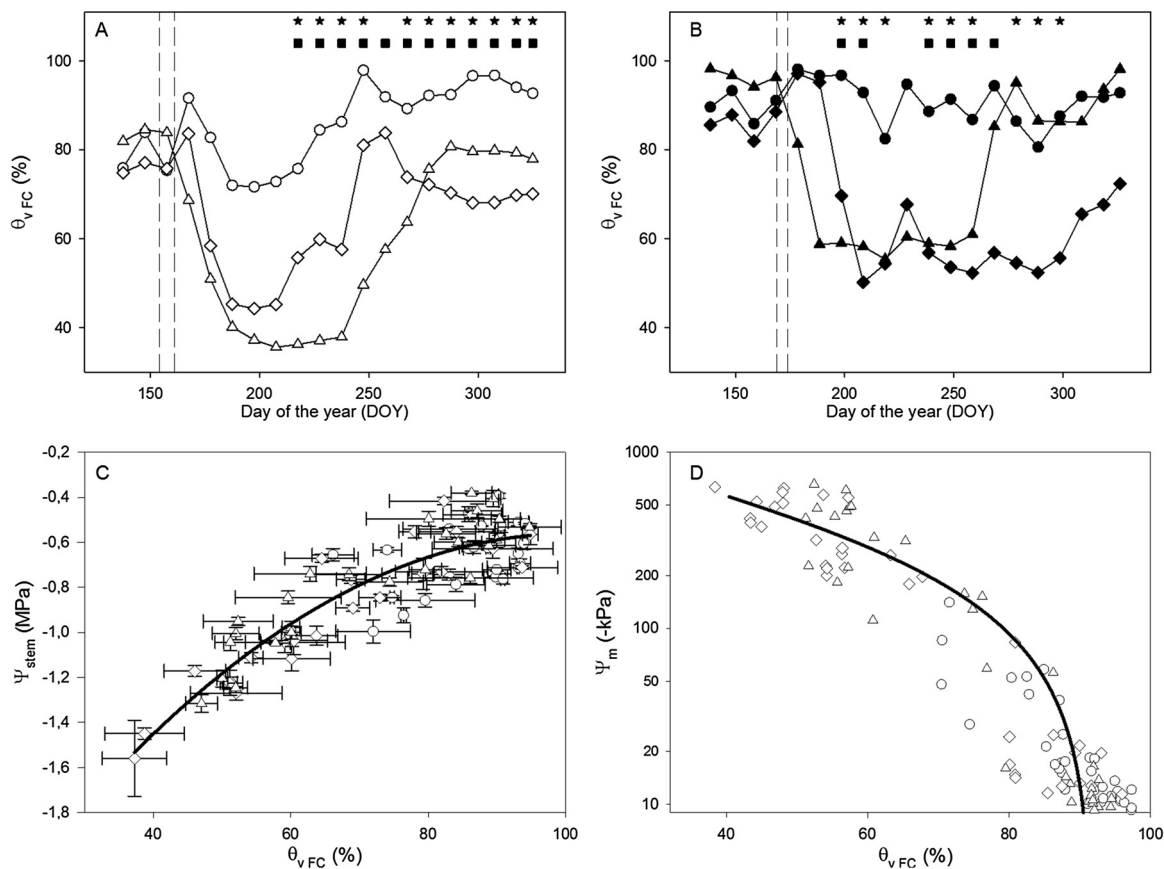


Fig. 3. (A and B) Seasonal evolution of stomatal conductance ( $g_s$ ) in 2015 and 2016, respectively. (C) Linear relationship between  $g_s$  and  $\Psi_{stem}$  expressed as difference between RDI and FRM (tri) respect to CTL. (D) Linear relationship between  $g_s$  and  $\Psi_{stem}$  during summer. Treatments: CTL, irrigated at 110%  $ET_c$  (circles); RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest (diamonds); FRM a treatment based on normal farming practices (triangles). Each point is the mean value of 6 measurements. \* and ■ denote significant differences between CTL and RDI and between CTL and FRM, respectively, according to ANOVA ( $P < 0.05$ ).



**Fig. 4.** (A and B) Seasonal evolution of soil volumetric water content referred to field capacity ( $\theta_{vFC}$ ) at 70 cm in 2015 and 2016, respectively. (C) Midday stem water potential relationship with the average soil volumetric water content at 20 and 40 cm depth for 2015 and 2016. (D) Relationship between mean values of ten days of soil matric potential and soil volumetric water content for 2015 and 2016. Treatments: CTL, irrigated at 110%  $ET_c$  (circles); RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest (diamonds); FRM a treatment based on normal farming practices (triangles). Each point is the mean value per replicate. \* and ■ denote significant differences between CTL and RDI and between CTL and FRM, respectively, according to ANOVA ( $P < 0.05$ ).

A relationship between  $\theta_{vFC}$  mean value at 20 and 40 cm depth and midday stem water potential was found ( $\Psi_{stem} = -0.00024 \theta_{vFC}^2 + 0.0484 \theta_{vFC} - 3.003$ ;  $R^2 = 0.81$ ; Fig. 4C). As might be expected,  $\Psi_m$  at 25 and 50 cm and  $\theta_{vFC}$  at 25 and 50 cm showed a good non linear relationship during both 2015 and 2016 ( $\Psi_m = 681.2 \ln \theta_{vFC} - 3078$ ;  $R^2 = 0.80$ ), which can be taken as confirmation of the reliability of the data obtained from both types of soil sensors (Fig. 4D).

### 3.6. Soil matric potential

The mean soil matric potential values at 25 and 50 cm depth were in line with the irrigation regimes. All the treatments showed mean  $\Psi_m$  values above  $-30$  kPa at 25 50 cm during preharvest, which reflects non limiting soil water conditions (Fig. 5A D). However, differences in  $\Psi_m$  between treatments appeared during postharvest. RDI and FRM reached very low values at both depths and in both years of the study (Fig. 5A D). A non linear association between  $\Psi_m$  mean values at 25 50 cm depth and  $\Psi_{stem}$  was found,  $\Psi_{stem} = 10^{-8} \Psi_m^3 + 10^{-5} \Psi_m^2 + 0.0045 \Psi_m - 0.4998$ ;  $R^2 = 0.71$  (Fig. 5E). This association confirmed, as expected, that plant water status is related to soil water availability in the root zone, and consequently to  $\Psi_m$ , as already indicated by numerous other authors.

### 3.7. Midday stem water potential estimation

As the plant responds to soil water availability as well as

atmospheric demand,  $\Psi_m$  and VPD were related to estimate  $\Psi_{stem}$ . This relation describes two different situations to obtain an estimated  $\Psi_{stem}$  value, which depends on soil water availability and evaporative demand. If  $\Psi_m$  is lower than  $-30$  kPa, there is a limiting soil water condition and the reference line is derived from  $\Psi_m$  and VPD, while if  $\Psi_m$  is higher than  $-30$  kPa,  $\Psi_{stem}$  is mainly influenced by the evaporative demand, so it can be estimated from VPD. The VPD best explained  $\Psi_{stem}$  changes under non water deficit conditions.

A model consisting of two situations described above was obtained to estimate  $\Psi_{stem}$  from the 2015 data. To validate this model,  $\Psi_{stem}$  values measured in 2016 were compared to the values estimated by the model. Estimated  $\Psi_{stem}$  values were able to explain 86% of the variation in the data measured (Fig. 6). Similarly, 2016 data were used to obtain a similar model, which was validated with 2015  $\Psi_{stem}$  data, thus explaining 80% of the data measured in 2015 (Fig. 6). The two regression lines obtained from the models showed no significant differences between them according to ANOVA, nor did intercepts  $P$  value = 0.94, or slopes  $P$  value = 0.31,  $R^2 = 0.82$  (Fig. 6). As both regression lines were similar, both years' data were used to obtain a multiple linear regression model for situation 1 (Eq. (1)), and a linear regression model for situation 2 (Eq. (2)).

$$\text{If } \Psi_m < -30 \text{ kPa } \Psi_{stem \text{ estimated}} = -0.3506 + 0.000642\Psi_m - 0.2143VPD \quad R^2 = 0.74; \quad P \text{ Value} < 0.01 \quad (1)$$

$$\text{If } \Psi_m > -30 \text{ kPa } \Psi_{stem \text{ estimated}} = -0.1674VPD - 0.3197 \quad R^2 = 0.78; \quad P \text{ Value} < 0.01 \quad (2)$$



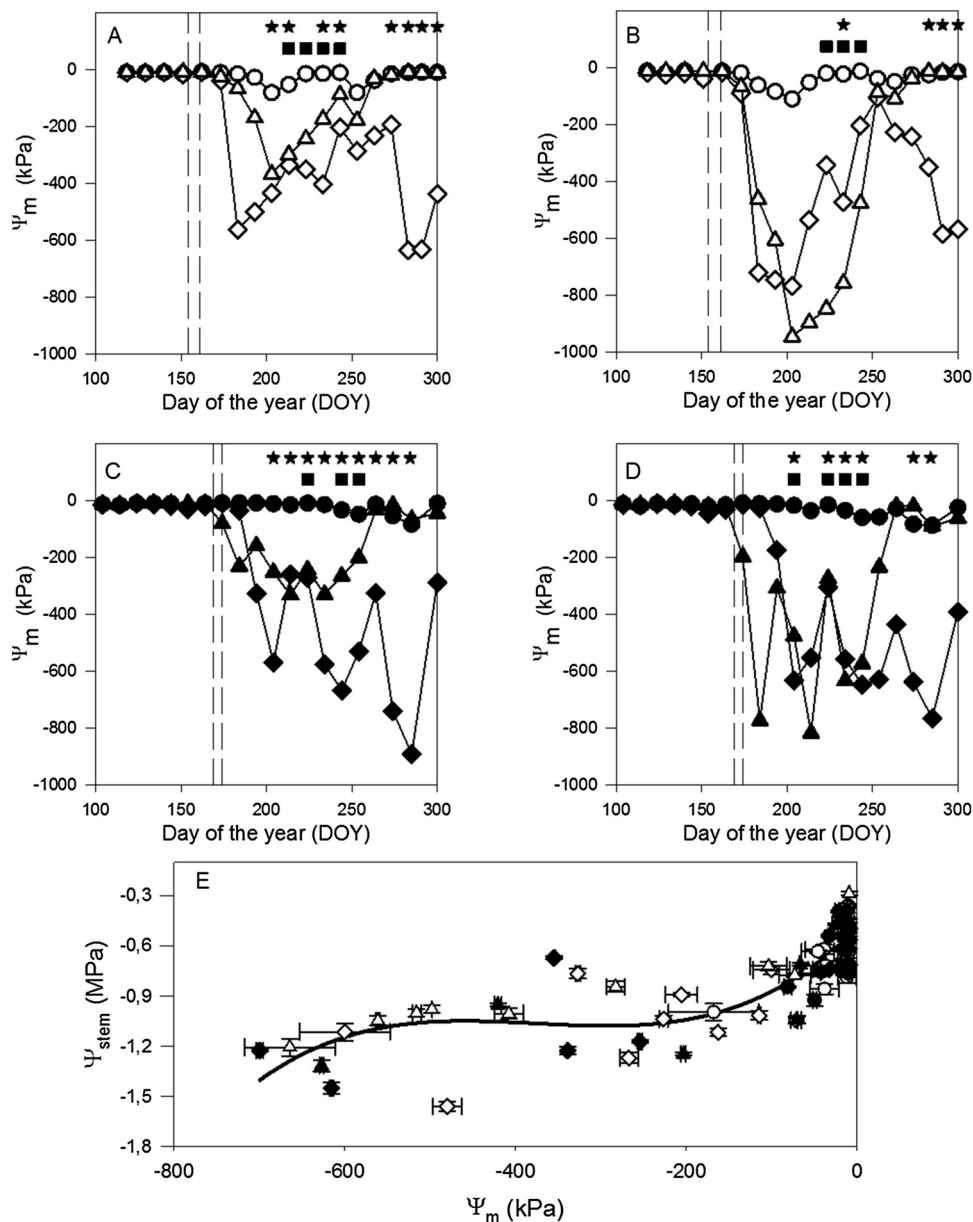


Fig. 5. (A and B) Seasonal evolution of soil matric potential ( $\Psi_m$ ) in 2015 at 25 and 50 cm depth, respectively. (C and D) Seasonal evolution of soil matric potential ( $\Psi_m$ ) in 2016 at 25 and 50 cm depth, respectively. (E) Midday stem water potential relationship of mean values with the soil matric potential 2015–2016. Treatments: CTL, irrigated at 110%  $ET_c$  (circles); RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest (diamonds); FRM a treatment based on normal farming practices (triangles). Each point is the mean value of 10 days per replicate. \* and ■ denote significant differences between CTL and RDI and between CTL and FRM, respectively, according to ANOVA ( $P < 0.05$ ).

### 3.8. Vegetative growth

Vegetative growth was measured as canopy volume in midsummer before the annual pruning, which was weighed (27th August). There were no differences among treatments in canopy volume or tree shaded area in 2015 or 2016 (Table 2). In contrast, there were differences among treatments in pruning weight. Pruning was performed in such a way that a similar canopy volume was restored to all the trees regardless of the water they received, which meant that the pruning weight differed each year of the study and cancelled differences in canopy volume. This weight was much higher in 2016, when it differed significantly between the three treatments (Table 2).

An inverse relationship was found between BGR, expressed as the sum of the daily percentage of growth, and the  $WSI_{\Psi_{stem}}$  during post harvest, the period when vegetative growth mainly takes place ( $\% BGR = -0.0007 WSI_{\Psi_{stem}} + 0.0856$ ;  $R^2 = 0.73$ ). In the same way, branch cross section annual increase, expressed as branch cross section relative growth rate (BCSGR) was also seen to be inversely related to  $WSI_{\Psi_{stem}}$  ( $BCSGR = -0.0014 WSI_{\Psi_{stem}} + 0.2373$ ;  $R^2 = 0.64$ ).

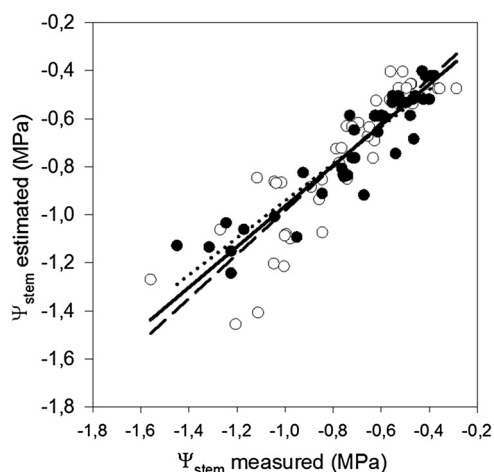
### 3.9. Yield

Despite the different amounts of water applied, no significant differences in fruit yield among treatments were found in 2015 or 2016. The year 2016 was a high cropping year ( $27 \text{ t ha}^{-1}$ ), with a level that was almost one and a half times that of 2015 ( $16.5 \text{ t ha}^{-1}$  plus  $1.8 \text{ t ha}^{-1}$  as a result of hail damage), with the result that fruit unitary weight was higher in 2015 ( $11.1 \text{ g}$ ) than in 2016 ( $8.8 \text{ g}$ ), with no differences among treatments.

### 3.10. Sensitivity of the indicators

Evaluation analysis showed  $g_s$  to be the plant indicator with the highest signal intensity (SI) in both years of the study followed by MDS and  $\Psi_{stem}$  (Table 3). Although  $\Psi_{stem}$  had a lower signal intensity than  $g_s$  and similar to that of MDS, its sensitivity values ( $S$  and  $S^*$ ) were much higher due to the low coefficient of variation (CV) obtained.

Soil water deficit indicators showed high SI values. The SI of  $\theta_{vFC}$  (data not shown) was similar, and even slightly lower than the SI of the plant indicators, but  $\Psi_m$  had the highest SI of all the studied indicators



**Fig. 6.** Regression equation obtained for measured and estimated stem water potential for 2015 (white circles- dashed line) and 2016 (black circles- dotted line). From both years 2015 and 2016 stem water potential measured and estimated, a regression equation was obtained (solid line –  $R^2 = 0.84$ ).

(Table 3). Soil indicators also had the highest CV. Thus,  $\Psi_m$  was the indicator with the highest sensitivity in spite of the high CV.

#### 4. Discussion

Seasonal stem water potential was obtained in three irrigation treatments over two consecutive years. The three patterns showed a similar evolution, with values decreasing as evaporating demand rose. However, RDI and FRM showed a steeper and longer falling slope than CTL, especially in RDI once the deficit was applied, minimum values in all of them occurring in midsummer (Fig. 2A and B). This steep drop could also be a sign that the soil water reservoir was limited to the zone wetted by the dripper, which suggests the low capacity of the not wetted zone to mitigate the reduction of water supply during post harvest, the deficit period. West et al. (1970) reported that the roots of the dry zone remain dormant in dry periods and are activated only after rain.

RDI and FRM treatments fell below  $-1.3$  MPa, the  $\Psi_{stem}$  threshold obtained from the polynomial relationship between  $\Psi_{stem}$  vs. MDS, on three occasions during postharvest (Fig. 2A and B). However, these treatments did not exceed the threshold value of  $-1.5$  MPa reported by Marsal et al. (2010) in sweet cherry var. Summit. Consequently, RDI and FRM plants did not suffer severe water stress and so avoided any effect on the sweet cherry yield and fruit quality the following season.

According to Girona et al. (2006), Naor and Peres (2001), Shackel et al. (2000),  $\Psi_{stem}$  can be considered as a reference water stress indicator in fruit trees. Therefore, we compared  $\Psi_{stem}$  with other indicators due to its high sensitivity, high signal intensity and the low CV

**Table 2**

Influence of irrigation treatment on the vegetative growth over two seasons (2015–2016). CTL, irrigated at 110%  $ET_c$ ; RDI, a regulated deficit irrigation treatment, which satisfied 100%  $ET_c$  at preharvest and floral differentiation and 55%  $ET_c$  at postharvest; FRM a treatment based on normal farming practices.

	Dry weight pruning wood kg tree <sup>-1</sup>		Canopy volume m <sup>3</sup>		Shaded area m <sup>2</sup>		BGR accumulate mm		BCS cm <sup>2</sup>	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
CTL	2.59b	4.39a	9.62	10.76	7.06	7.75	8.60	15.85a	103.87	117.38
RDI	2.04b	2.87c	9.98	10.05	7.07	7.42	5.44	8.96b	79.46	85.01
FRM	3.74a	3.68b	10.45	11.43	7.33	7.99	5.53	10.31b	89.98	98.05
ANOVA	*	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.

BGR (branch growth rate) accumulate and BCS (branch cross section) values are the mean of the trees with dendrometer (2 per replicate) and pruning wood, canopy volume and shaded area values are the mean of five trees per replicate. n.s. indicates no significant differences among treatments. Mean values followed by different letters in the same column show significant differences among treatments according to the Tukey's test, \* denotes  $P < 0.05$ , \*\* denotes  $P < 0.01$ .

value (Table 3). The need for a comparative assessment of common water stress indicators on the part of researchers, technicians and farmers led us to study different correlations of  $\Psi_{stem}$  with  $g_s$ , MDS,  $\theta_v$  and  $\Psi_m$ .

The seasonal evolution of stomatal conductance ( $g_s$ ) showed good correlation with stem water potential ( $R^2 = 0.68$ ), similar to that reported by Marsal (2012) in 'Summit' sweet cherry (Fig. 3D). Stomatal conductance was the plant indicator with highest signal intensity (Table 3), as reported by de la Rosa et al. (2014) in early nectarine trees. These results were similar to those obtained in apple, grapefruit and nectarine by Naor (1998) and in plum by McCutchan and Shackel (1992). During postharvest, differences among treatments appeared as different irrigation treatments were imposed. We found no differences in mean preharvest  $g_s$  values between 2015 and 2016, despite the great difference in fruit load, which could mean that  $g_s$  in sweet cherry var. Prime Giant did not differ with fruit load. Likewise, Naor (2001) did not observe any influence of different fruit loads on  $g_s$  in pear trees, contrary to that reported in almond trees by Puerto et al. (2013) and in apple trees by Wünsche et al. (2000). In our case, the lack of differences in  $g_s$  between years could be explained by differences in unitary fruit weight, 11.1 g in 2015 when total yield was  $16.5 \text{ t ha}^{-1}$  and 8.8 g in 2016 when total yield was  $27 \text{ t ha}^{-1}$ .

Moreover, mean postharvest  $g_s$  values in 2015 were slightly higher than in 2016 in all treatments, which could be due to a 7% greater VPD values in 2016 on the days when measurements were made (Fig. 3A and B). Thus, Flore (1985) pointed out in sour cherry a similar stomatal response to VPD variations.

MDS showed a more similar S to  $g_s$  than to  $\Psi_{stem}$  and  $\Psi_m$ . However, MDS was the plant indicator showing the highest correlation with  $\Psi_{stem}$  ( $R^2 = 0.78$ ). Moreover, once the deficit irrigation was applied, MDS showed significant differences between treatments ten days before  $\Psi_{stem}$  did so. In 'Prime Giant', MDS would allow early water stress detection during the most water sensitive moments, such as fruit set and development and flower induction and differentiation, when water stress may reduce fruit growth or decrease the following year's harvest. This suggests that MDS is more useful than other water indicators for detecting slight water deficits during phenological stages when plant water stress should be avoided. Moreover MDS has the advantage that its measures can be automated. The data obtained for both years showed that the  $\Psi_{stem}$  and MDS relationship changed throughout the growing season (Fig. 2E). For example, the relationship varied with the physiological phase of the crop and the age of the tree tissues, similarly to that reported in pomegranate by Intrigliolo et al. (2011) and in peach by Mirás Avalos et al. (2016). In our case, the best relationship was found during the final fruit growth and early postharvest periods (Fig. 2F). Intrigliolo et al. (2011) reported that changes in the MDS and  $\Psi_{stem}$  relationship could be attributed to tissue elasticity changes since it is known that older tissues are less elastic; hence, as the season progresses, MDS could lose its sensitivity, especially towards the end of the season (Tyree and Jarvis, 1982). When data were pooled, it was

**Table 3**  
Sensitivity analysis of the water stress indicators studied in RDI and FRM treatments during postharvest.

	$\Psi_{\text{stem}}$				$g_s$				MDS				$\Psi_m$			
	2015		2016		2015		2016		2015		2016		2015		2016	
	RDI	FRM	RDI	FRM	RDI	FRM	RDI	FRM	RDI	FRM	RDI	FRM	RDI	FRM	RDI	FRM
SI	1.52	1.29	1.46	1.41	1.63	1.55	2.09	2.70	1.62	1.45	1.52	1.30	11.21	10.35	17.73	11.62
CV	0.09	0.08	0.06	0.08	0.20	0.22	0.21	0.22	0.28	0.21	0.16	0.27	0.44	0.46	0.32	0.70
S	17.32	16.63	23.22	16.85	8.04	7.16	10.06	12.47	5.75	6.92	9.47	4.91	25.61	22.54	55.04	16.71
S*	5.90	3.75	7.34	4.88	3.10	2.55	5.26	7.86	2.21	2.14	3.23	1.14	23.32	20.36	51.94	15.27

Water stress indicators:  $\Psi_{\text{stem}}$  (midday stem water potential),  $g_s$  (stomatal conductance), MDS (maximum daily branch shrinkage) and  $\Psi_m$  (soil matric potential). SI means signal intensity and CV coefficient of variation. S refers to sensitivity according to Goldhamer and Fereres (2001) and S\* to de la Rosa et al. (2014).

necessary to establish as threshold value the limit below which  $\Psi_{\text{stem}}$  and MDS are not directly related, i.e. when the slope changes direction (inflection point). According to our data, this limit could be established as  $-1.3$  MPa, which corresponded to a maximum branch shrinkage of  $500 \mu\text{m}$ . Determining the values between which MDS is related to  $\Psi_{\text{stem}}$  in sweet cherry var. Prime Giant is important in order to set MDS irrigation deficit scheduling (Girón et al., 2016). MDS values were less stable than  $\Psi_{\text{stem}}$  within the same treatment, which agrees with the results reported in almond trees (Fereres and Goldhamer, 2003), apple (Naor and Cohen, 2003), and plum (Intrigliolo and Castel, 2006). The CV of MDS was almost three times higher than the CV obtained for  $\Psi_{\text{stem}}$ .

These results point to MDS as one of the earliest water stress indicators in sweet cherry trees, as reported for plum trees (Intrigliolo and Castel, 2004) and lemon trees (Ortuño et al., 2004). This rapid stress detection capacity might make MDS a useful scheduling tool for avoiding detrimental stress levels in RDI treatments during preharvest, the period of fruit development, particularly in early varieties such as Prime Giant. However, during postharvest it would be less useful than  $\Psi_{\text{stem}}$ , especially during July and August (DOY 185–245 in our study). Despite this,  $SI_{\text{MDS}}$  could also represent an interesting water stress indicator for RDI management depending on the different phenological phases in sweet cherry trees.

As expected,  $\theta_v$  showed good relationship with  $\Psi_{\text{stem}}$  ( $R^2 = 0.81$ , Fig. 4C). This polynomial relationship was slightly higher than the correlation reported by Livellara et al. (2011) in young sweet cherry trees var. Brooks, and indicates that  $\theta_v$  not only strongly influences plant water status but is also closely related with it. This correlation, similar to that the one obtained between  $\Psi_m$  and  $\Psi_{\text{stem}}$ , points to the importance of soil monitoring in irrigation scheduling to prevent or cause soil water deficits and also to obtain threshold values, as many authors, such as Thompson et al. (2007), have reported. This correlation between  $\Psi_{\text{stem}}$  and both soil indicators, and between themselves, is not univocal, since it is slightly different if it is obtained during the soil water absorption or desorption process (Fredlund and Xing, 1994). However, the sensitivity analysis highlighted  $\theta_v$  as the indicator with the lowest sensitivity, due to the high noise rather than low signal intensity. This may have been related with the difficulty involved in placing the FDR probe in a stony soil, compared to an MPS 6 sensor whose installation does not require a probe.

Of the parameters analyzed in the sensitivity analysis,  $\Psi_m$  had the highest signal intensity and sensitivity. However, in a soil of medium water retention capacity, such as ours, small decreases in the soil volumetric water content below field capacity cause significant decreases in  $\Psi_m$ , which become pronounced as the soil water is decreasing. For this reason, the high coefficient of variation obtained should be taken into account as a disadvantage. Variability in the  $\Psi_m$  readings increased with decreasing  $\Psi_m$  values, meaning that replications are necessary to obtain a more accurate soil water status representation and to reduce variation (Naor, 2004).

Irrigation management using  $\Psi_m$  sensors is based on the use of

recommended threshold  $\Psi_m$  values to indicate when irrigation is required (Shock and Wang, 2011), but  $\Psi_m$  thresholds are influenced by the phenological stage and the evaporative conditions (Hanson and Peters, 2000; Lou et al., 2016). In this respect, and on the basis of the good results obtained with  $\Psi_m$  (high SI and S and closely related to  $\Psi_{\text{stem}}$ ,  $R^2 = 0.71$ ) in soil water deficit conditions ( $\Psi_m < -30$  kPa), the  $\Psi_m$  values were used to estimate  $\Psi_{\text{stem}}$ . The good relationship between the estimated  $\Psi_{\text{stem}}$  and the  $\Psi_{\text{stem}}$  measured throughout the growing season ( $R^2 = 0.84$ ) can be explained by efficient water movements in the soil and within the tree when evaporative demand increases, which under our conditions was mainly due to a hydraulic control phenomenon (Sdoodee and Somjun, 2008).

$\Psi_{\text{stem}}$  is a very reliable and sensitive measure to determine plant water status, the estimation of which would otherwise involve a very laborious process; therefore an equation relating  $\Psi_m$  measured at midday and daily VPD with  $\Psi_{\text{stem}}$  was obtained for trees under soil water limiting conditions ( $\Psi_m < -30$  kPa). This estimation is interesting because both parameters are easily available to growers and the data used are the direct real time measurements, not converted as the MDS. So farmers or technicians who already have soil and meteorological sensors installed in their orchard, many more than those who use MDS, have the possibility of directly estimating  $\Psi_{\text{stem}}$ . Such estimation based on different soil and meteorological variables, depend on the crop evaporative demand, as well as on the soil water status. Soil indicators showed a better relation with  $\Psi_{\text{stem}}$  (Figs. 4C and 5E) than meteorological variables (Table 1) and made a higher contribution to  $\Psi_{\text{stem}}$  estimation in deficit treatments and in the multiple linear regressions with both types of variables used (climate and soil). This  $\Psi_{\text{stem}}$  and  $\Psi_m$  relationship showed that  $\Psi_{\text{stem}}$  was clearly influenced by  $\Psi_m$ , meaning that the obtained equation remained stable for longer periods and for different irrigation treatments. These results reflected the importance of the soil components as reported by Abrisqueta et al. (2015) in peach trees and Martí et al. (2013) in orange trees. Eqs. (1) and (2) can be useful tools for  $\Psi_{\text{stem}}$  estimation since this parameter is not totally dependent on environmental indicators (Stagno et al., 2011). Thus, this study points to the importance of knowing the soil water status when estimating  $\Psi_{\text{stem}}$ .

The goodness of fit of the calculated relations between different indicators and  $\Psi_{\text{stem}}$  can be ordered:  $MDS = \theta_v > \Psi_m > g_s$ . But according to the sensitivity analysis, the indicators could be classified as:  $\Psi_m > \Psi_{\text{stem}} > g_s > MDS > \theta_v$ . Sensitivity was measured according to Goldhamer and Fereres (2001), and de la Rosa et al. (2014) (S and S\*, respectively). For all the indicators measured S\* was smaller than S, although the order of the indicators did not change.  $\Psi_{\text{stem}}$  values were more stable, with a lower coefficient of variation, than  $\Psi_m$ . Soil matric potential values reflected greater fluctuations than stem water potential which matches with the higher SI of  $\Psi_m$  and the results reported by Naor (2004) in plum trees. Moreover,  $\Psi_{\text{stem}}$  had greater sensitivity than  $g_s$  in all treatments and both years of the study, and  $\Psi_{\text{stem}}$  was twice as sensitive as  $g_s$  when measured as S, and 33% more sensitive when measured as S\*. This is because S is more dependent on the coefficient

of variability. These results are in line with those of Abdelfatah et al. (2013) in young sweet cherry trees. The variability observed in  $g_s$  and MDS has been reported in other tree species of the genus *Prunus*, such as nectarine (de la Rosa et al., 2014) and plum (Intrigliolo and Castel, 2004) and it could be explained by the high variability of the measurements made in sun exposed leaves and the fact that branch shrinkage is not only affected by the tree water status but also by other anatomical characteristics (Ballester et al., 2014). The high SI and CV obtained for soil indicators compared to plant indicators agree with the results reported in olive trees under RDI by Agüero Alcaras et al (2016). Likewise, as Castel and Buj (1990) reported, soil indicators showed the highest CV.

Relationships between vegetative growth (measured as branch growth) and the water stress integral for both years of the study underline the influence of the irrigation treatments on vegetative growth. BGR was lower in FRM and RDI than in CTL during the 2016 post harvest period when deficit irrigation was applied. Likewise, RDI pruning wood was statistically lower than CTL. It is well known that plant or organ growth is a process that is highly sensitive to water stress (Hsiao, 1973).  $WSI_{\Psi_{stem}}$  was similar in 2015 and 2016 since the water applied and meteorological parameters were similar. Although fruit yield did not differ significantly among irrigation treatments, it was observed that sweet cherry unitary weight was influenced by crop load, as reported by Einhorn et al. (2011). Although the different treatments had no effect on fruit yield, the water supplied in RDI was 40 and 33% lower than in CTL and FRM, respectively, during both years of the study.

## 5. Conclusion

The results obtained in the search for an overall indicator for use in irrigation management suggest the following order:  $\Psi_{stem} > \Psi_m > MDS > g_s > \theta_v$ .  $\Psi_{stem}$  was seen to be the most reliable and stable water stress indicator as it clearly detected irrigation changes. The main drawback with  $\Psi_{stem}$  is that the measurement process cannot be automated and it provides one off measurements. Thus, we propose a  $\Psi_{stem}$  estimation model based on two easily available parameters, VPD and  $\Psi_m$ , which continuously register soil and atmosphere water status and obtain indirectly information on the plant water status. However, two situations were distinguished, depending on the soil water status: if  $\Psi_m$  is higher than  $-30$  kPa (no stress),  $\Psi_{stem}$  can be estimated from VPD alone, while if  $\Psi_m$  is lower than  $-30$  kPa (mild moderate stress),  $\Psi_{stem}$  should be calculated using both  $\Psi_m$  and VPD. In our experiment, the estimated  $\Psi_{stem}$  explained 84% of the variation of the measured  $\Psi_{stem}$ . The resulting estimation of  $\Psi_{stem}$  could help know the current plant water status during the growing season and act as a useful tool in irrigation decision making

MDS was the indicator that first detected water stress, which makes it useful in conditions when a slight water deficit can affect vegetative and fruit growth and yield. An MDS vs.  $\Psi_{stem}$  regression showed a threshold value of  $-1.3$  MPa. So MDS as water stress indicator only has a limited range in which it can be used to manage RDI in 'Prime Giant' sweet cherry.

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Article II

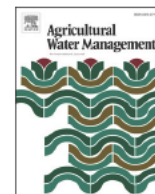
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# Water relations and quality changes throughout fruit development and shelf life of sweet cherry grown under regulated deficit irrigation



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## ABSTRACT

The performance of 'Prime Giant' sweet cherry trees under three different irrigation regimes was examined during two consecutive seasons, 2015–2016 and 2016–2017. The irrigation treatments evaluated were: (i) a control treatment (CTL) irrigated at 110% of crop evapotranspiration ( $ET_{cd}$ ) to maintain non-limiting soil water conditions, (ii) RDM a regulated deficit irrigation treatment which applied a mild water stress during preharvest (90% of  $ET_{cd}$ ) and a medium stress during postharvest (65% of  $ET_{cd}$ ) and (iii) RDS a regulated deficit irrigation treatment which applied a severe water stress (55% of  $ET_{cd}$ ) during postharvest.

There were significant differences during postharvest in soil and plant water indicators such as soil matric potential, midday stem water potential and maximum daily branch shrinkage between CTL and regulated deficit irrigation treatments (RD). However, only midday stem water potential was able to distinguish between RDM and CTL during preharvest. Fruit quality parameters such as fruit size, color, soluble solids concentration (SSC) were periodically measured during fruit developing as well as fruit water potential and osmotic fruit water potential. In 2016, there were no differences in any parameter between treatments. However in 2017 both deficit treatments led to fruits with a higher SSC and darker color than CTL. There were no differences in fruit yield or number of fruits per tree among treatments in either year of the study. When fruit quality was assessed in 2016, the color of the pedicels of fruits from the deficit irrigation treatments were greener than those from CTL after 20 days of cold storage at 2 °C and 90% relative humidity (RH) but and after 5 days of shelf-life simulation (15 °C and 65% RH) the differences between treatments disappeared.

## 1. Introduction

Sweet cherry (*Prunus avium* L.) is a high valuable cash fruit crop widely appreciated by consumers. Its cultivation requires lower amounts of water than other fruit trees (García Montiel et al., 2010) which is important in areas where water is often a limiting factor for production. According to CIHEAM (2010), the Mediterranean Basin is a highly productive region very susceptible to climate change and the loss of water resources. Mediterranean agriculture, which is the biggest water consuming activity of the whole area, needs to face up to this situation and adopt not only suitable technological tools but also water saving strategies to increase crop water use efficiency (Costa et al., 2007; Martínez Ferri et al., 2013).

Regulated deficit irrigation (RDI) is a water saving strategy based on supplying reduced irrigation doses in specific periods, depending on the

phenology of the crop, which will not negatively affect final yield or fruit quality and should decrease excessive vegetative growth (Chalmers et al., 1981; Mitchell and Chalmers, 1982). RDI has been studied with positive results in different deciduous tree crops (Behboudian and Mills, 1997), and although RDI strategies have also been applied in sweet cherry (Dehghanisanij et al., 2007; Marsal et al., 2009, 2010; Nieto et al., 2017), there is little information available on the effect of deficit irrigation on sweet cherry water relationships, growth and quality not only at harvest but also after a simulated cold storage period and a complementary shelf life.

Sweet cherry is one of the most popular temperate fruits of the genus *Prunus* and quality parameters such as size and color (visual appearance) and its sugar acid flavor play an important role in its wide acceptance (Crisosto et al., 2003). This study was conducted on sweet cherry 'Prime Giant', known to be a highly appreciated cultivar by

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**Table 1**

Mean seasonal values (April – November) of reference crop evapo-transpiration ( $ET_0$ ), precipitation, irrigation water applied and reduction (%Red) compared to control treatment (CTL) for the different deficit irrigation treatments (RDM and RDS) and irrigation periods, preharvest (Pre), floral differentiation (Floral Diff) and postharvest (Post).

	Floral Diff 2015	Post 2015	%Red	Pre 2016	%Red	Total 2015-2016	%Red	Floral Diff 2016	Post 2016	%Red	Pre 2017	%Red	Total 2016-2017	%Red	Total mean	SE	%Red
$ET_0$ (mm)	109	655		357		1121		115	573		366		1053		1087	34	
Precipitation. (mm)	13	109		84		206		0	238		110		348		277	71	
Irrigation water applied (mm)																	
CTL	57	437		214		708		67	422		190		679		694	15	
RDM	55	225	48	175	18	455	36	67	221	48	151	21	439	35	447	8	36
RDS	57	182	58	191	11	430	39	65	168	60	166	13	399	41	414	15	40

consumers for its size, mahogany color, high firmness and balanced flavor (Díaz Mula et al., 2009).

However, sweet cherry is reported to be a non climacteric fruit, is very perishable so there is a need of adequate postharvest techniques like temperature and relative humidity management (Habib et al., 2015; Serrano et al., 2005). Fruit decay, softening, off flavors and particularly pedicel browning, due to water loss, are typical quality disorders suffered by sweet cherries during cold storage and subsequent shelf life, which may lead to rejection on the part of consumers (Bernalte et al., 2003). Thus, an evaluation of such quality attributes during fruit growth, at harvest and during retail conditions will provide information to assess whether the irrigation strategy applied could have influenced fruit development, yield, or quality at any time 'from farm to fork'. Spain is the fifth sweet cherry producer in the world (FAOSTAT, 2015) and although its production is mainly sold for domestic consumption and for export to nearby European countries, they are also sent by sea to more distant countries, a journey that may take 20–30 days. Sweet cherry has been reported to suffer rapid quality deterioration and decay during storage and their subsequent shelf life (Habib et al., 2015) as a result of multiple factors such as harvest time, storage conditions, cultivar, etc. There is little on how irrigation management could affect the evolution of 'Prime Giant' quality.

Therefore, the aim of this work was to assess the effects of deficit irrigation on the plant and fruit water relations, fruit growth, yield and physicochemical characteristics at harvest and after cold storage and during subsequent retail conditions in 'Prime Giant' sweet cherries. For this, the physiology and quality of 'Prime Giant' sweet cherries grown under two different regulated deficit irrigation strategies and one well watered control were compared in order to improve water productivity and recommend the best irrigation strategy in areas where water is a limited resource.

## 2. Materials and methods

### 2.1. Experimental site, plant material and treatments

The study was conducted in a 0.5 ha commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W) during two consecutive growing seasons, 2015–2016 and 2016–2017. The soil has a sandy loam texture, is moderately stony with a normal active limestone (2.7%), low potassium (0.32 meq 100 g<sup>-1</sup>) and high available phosphorus (108.67 mg kg<sup>-1</sup>) content. The irrigation water, drawn from a well, was of low salinity (electrical conductivity,  $EC_{25} = 0.8$  dS m<sup>-1</sup>). The climate is typically Mediterranean, with low rainfall distributed in autumn and spring, hot dry summers and mild winters. Average annual reference evapotranspiration was about 1250 mm and average rainfall varied from 250 to 360 mm.

The plant material consisted of fifteen year old sweet cherry trees (*Prunus avium* L.) 'Prime Giant', grafted on SL64 rootstock, and 'Early Lory' and 'Brooks' as pollinizers, and spaced at 5 m × 3 m. At the beginning of the experiment, the vase shaped trees had an average trunk

diameter of 16.3 ± 0.2 cm, a canopy volume of 10.5 ± 0.4 m<sup>3</sup> and ground cover of around 55%. Trees were drip irrigated using a single drip line for each tree row, with three pressure compensated emitters per tree, each with a discharge rate of 4 L h<sup>-1</sup>.

Full bloom was in late March–early April, day of year (DOY) 99 and 80, in 2016 and 2017 respectively, and fruit was harvested 70 and 73 days after full bloom (DAFB), (on June 17<sup>th</sup> in 2016 and on June 2<sup>nd</sup> in 2017).

The different irrigation treatments were initiated each season before flowering and suspended at the end of November. Horticultural practices (e.g. fertilization, weed control and pruning) were the same for the trees of all treatments and were carried out by the technical department of the commercial orchard. Fertilization was applied through the irrigation system with the water and was the same in all treatments despite the amount of the water applied. The fertilization program consisted of 63, 30, 107 and 8 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and CaO, respectively.

Three irrigation treatments were applied: a control (CTL) irrigated at 110% of crop evapotranspiration ( $ET_{cd}$ ) to maintain non limiting soil water conditions and two regulated deficit irrigation treatments (RD): (i) RDM irrigated at 90% of  $ET_{cd}$  during preharvest (mild water stress critical period), 100% of  $ET_{cd}$  during flower differentiation (15–20 days after harvest–critical period) and 65% of  $ET_{cd}$  during postharvest (non critical period); (ii) RDS, a regulated deficit irrigation treatment which applied a severe water stress during postharvest, irrigated at 100% of  $ET_{cd}$  during preharvest and flower differentiation and 55% of  $ET_{cd}$  during postharvest.

Crop water requirements under drip irrigation ( $ET_{cd}$ ) were calculated using the following equation:  $ET_{cd} = ET_0 \times K_c \times K_p$ , where  $ET_0$  is reference evapotranspiration,  $K_c$  is a crop specific coefficient for sweet cherry reported by Marsal (2012) and  $K_p$  is a localization factor (Feres et al., 1982) related to the percentage of ground covered by the crop. The average annual amount of irrigation water applied was 694, 447 and 414 mm for CTL, RDM and RDS, respectively (Table 1).

Treatments were distributed according to a completely randomized block design with four replicates. Each replicate consisted of seven trees. The central five trees were used for experimental measurements, with the other served as guard trees. Sensors were installed in the two central trees or within their area of influence.

### 2.2. Water status measurements

The plant water status was measured every seven–ten days by measuring midday stem water potential ( $\Psi_{stem}$ ) at noon under field conditions according to the methodology proposed by McCutchan and Shackel (1992) with a Scholander pressure chamber (Model 3000, Soil Moisture Equipment, CA, USA) in six sunny, healthy and mature leaves located close to the trunk per irrigation treatment, two leaves per replicate in three replicates. On the same days, fruit water potential ( $\Psi_{fruit}$ ) was measured in six fruits per treatment. Fruit osmotic potential ( $\Psi_{nfruit}$ ) was measured in the same picked fruit as used to measure  $\Psi_{fruit}$ . After measuring  $\Psi_{fruit}$  in the field, these fruits were covered with



aluminum foil, labeled and immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Subsequently, in the laboratory  $\Psi_{\text{fruit}}$  was measured using a vapour pressure osmometer (Wescor 5600, Logan, UT, USA). Estimated fruit turgor potential ( $\Psi_{\text{fruit}}$ ) was obtained as the difference between osmotic and fruit water potential according to Milad and Shackel (1992).

Branch diameter variations were recorded by two dendrometers (LVDT sensors, model DF  $\pm 2.5$  mm, accuracy  $\pm 10$   $\mu\text{m}$ , Solartron Metrology, Bognor Regis, UK) per replicate, each on a main tree branch away from direct sunlight. Sensors were installed on aluminum and invar holders to prevent thermal expansion. The maximum daily branch shrinkage (MDS) was calculated as the daily difference between the maximum and the minimum branch diameter. The soil matric potential,  $\Psi_m$ , was measured by means of two thermal compensation capacitive sensors per replicate (MPS 6, Decagon Devices, Inc., Pullman, WA 99163 USA) at 25 and 50 cm depth and at a distance of 0.23 m from the emitter and 1.5 m from the trunk, under the canopy projection. Daily mean  $\Psi_m$  value from 11:00 to 14:00 h (solar time) was calculated from the data of both depths. Continuous measures of branch diameter fluctuations and matric potential were recorded every 30 s and the datalogger was programmed to report means every 10 min. Two replicates per treatment were equipped with a wired platform of one datalogger and two multiplexers (Campbell Scientific, Logan, USA) while the other two replicates used a wireless sensor network (WSN, Widhoc SS, Fuente Álamo, Spain) a ZigBee protocol system with six end devices, two per treatment, one router for coverage improvement, and one coordinator device that manages the network and records all the data. All the devices were connected using a star topological configuration. Data communication was performed using a point to point radio link provided by a local internet supplier. The data registered in the datalogger and ZigBee coordinator were stored in a cloud server placed in the university installations from where they could be downloaded anytime. Daily agrometeorological data were recorded by a weather station near the experimental orchard owned by the Spanish Agroclimatic Information Service (SIAR; <http://crea.uclm.es/siar/datmeteo/>).

### 2.3. Fruit growth characterization

In order to evaluate the irrigation effects on fruit growth and quality, ten representative sweet cherries were periodically picked per replicate, forty per treatment, every seven ten days during preharvest, fruit development. Non destructive measurements such as size and color were made on all fruits, forty fruits per treatment; destructive measurements were made on half of the fruits, twenty fruits per treatment were used in the determinations of fruit unitary dry weight and the other twenty fruits per treatment were used in the chemical analysis. Fruit size, equatorial and polar diameters (mm) were measured with a digital caliper (model 17 262, Acha, Eibar, Spain), and, from the data obtained, fruit volume ( $\text{cm}^3$ ) was calculated, equating 'Prime Giant' cherry shape as an ellipsoid oblate. Fruit and pedicel color was recorded on fruits and pedicels using a colorimeter (CR 400, Minolta, Tokyo, Japan) and illuminant D65. Lightness and hue angle ( $\text{hue}^{\circ}$ ) were obtained from the  $L^*$ ,  $a^*$  and  $b^*$  values of the CIE Lab scale system used. Fresh unitary weight (g) was weighed on an electronic balance (model AX623, Sartorius, Göttingen, Germany). Similarly to the fresh unitary weight, dry weight (g) was measured from the same fruits, which were dried at  $60^{\circ}\text{C}$  until constant weight in a ventilated oven (model Digiheat, JP Selecta, Barcelona, Spain). Growth rate (GR,  $\text{g d}^{-1}$ ) was calculated according to Grossman and DeJong (1995). In the chemical analysis, fruit was squeezed, as previously described Conesa et al. (2015), to determine from the juice of the fruit the soluble solids concentration (SSC, %) with a digital hand held refractometer (N1, Atago, Tokyo, Japan) at  $20^{\circ}\text{C}$ .

### 2.4. Fruit quality analysis at harvest

At both 2016 and 2017 harvests, all the fruit produced per tree were harvested from the 5 central trees of each replicate, and weighed in order to obtain fruit yield. To estimate fruit load, the number of fruits in 5 kg lots was counted in 3 trees per replicate, 12 trees per treatment to determinate fruit unitary weight. The number of cracked cherries in the sample was recorded to estimate cracking incidence per treatment. Fruit load (number of fruits per tree) was calculated from yield ( $\text{kg tree}^{-1}$ ) and fruit unitary weight (g) measured in the field. From the harvested fruit, twenty fruits from four replicates (eighty fruits per treatment) were collected to evaluate their quality. The same quality determinations periodically measured in the fruit during fruit development, such as size, color and SSC determination, were repeated at harvest in the twenty fruits selected. Apart from the above measurements, fruit and pulp firmness and titratable acidity were also evaluated.

Fruit and pulp firmness (N) were measured using a texture analyzer (CT3 Texture Analyzer, Ametek Brookfield Engineering Laboratories, MA, USA) in twenty fruits per replicate. Fruit firmness was the peak force measured during a puncture test in the equatorial region made by a 2 mm diameter cylindrical stainless probe which travelled until the skin broke at a test speed of  $10 \text{ mm s}^{-1}$ . Pulp firmness was similarly measured in the same cherries in a region of the fruit from which the skin had previously been removed.

The juice of ten cherries per replicate was extracted by a hand press squeezer and from this sample SSC, titratable acidity (TA; g malic acid  $100 \text{ mL}^{-1}$ ) and maturity index (MI) calculated as the rate between SSC and TA, were assessed. TA was measured with a titration (model 716 DMS Titrimo, Metrohm, Herisau, Switzerland) and calculated from the volume of NaOH (0.1 M) needed to reach a pH of 8.1 from a diluted juice sample of 5 mL of cherry juice and 45 mL of distilled water.

### 2.5. Fruit quality after cold storage and shelf life

For the postharvest trial, in 2016, 200 g of sweet cherries with similar ripening stage (size and color) per replicate, and three replicates per treatment, were selected from the first picking (June 17<sup>th</sup>). The cherries were stored in a rectangular plastic bowl of 0.75 L volume. Samples were maintained at  $2^{\circ}\text{C}$  and 90% relative humidity (RH) for 20 days and a subsequent retail period of 5 days at  $15^{\circ}\text{C}$  and 65% RH (shelf life conditions). Fruit quality was evaluated at harvest (day 0), after cold storage (day 20) and after shelf life simulation (day 20 + 5). At these times, equatorial and longitudinal diameters, fruit volume, fruit and pulp firmness fruit and pedicel color, SSC, TA and MI were measured in twenty cherries per replicate, and three replicates.

## 3. Results

### 3.1. Water applied

The climatic conditions over the study period were characteristic of Mediterranean semi arid areas. Reference crop evapotranspiration ( $\text{ET}_0$ ) was similar in 2015, 2016 and 2017. Annually, this amounted to 1250 mm, of which 1087 mm corresponded to the irrigation season (April–November); however, seasonal precipitation varied from 206 mm in 2015–2016 to 348 mm in 2016–2017. The mean water amounts applied in the seasons 2015–2016 and 2016–2017 were 694 (CTL), 447 (RDM) and 414 mm (RDS) (Table 1). During preharvest, RDM provided the least water (approximately 20% less than CTL), while during postharvest, RDS provided the least (59% less than CTL), while the water saved in RDM was 48% (Table 1).

### 3.2. Water stress indicators and water relations

The soil matric potential mean values were reflected the irrigation regimes

**Table 2**

Mean values of soil matric potential ( $\Psi_m$ ) and maximum daily shrinkage (MDS) for each irrigation period, preharvest (Pre), floral differentiation (Floral Diff) and postharvest (Post) in 2015, 2016 and 2017 for the irrigation treatments control (CTL), and both regulated deficit irrigation treatments, mild preharvest and medium postharvest deficit irrigation (RDM) and severe postharvest deficit irrigation (RDS).

		2015			2016			2017
		Floral Diff	Post	Pre	Floral Diff	Post	Pre	
$\Psi_m$	(kPa)	CTL	13.93	26.30a	12.54	16.79	28.00a	17.29
		RDM	11.24	242.17b	17.37	13.05	281.01b	64.79
		RDS	16.70	300.31b	13.24	22.45	324.30b	31.00
		ANOVA	n.s.	**	n.s.	n.s.	**	n.s.
MDS	( $\mu\text{m}$ )	CTL	154.44	161.96a	156.22	183.17	162.55a	149.85
		RDM	162.07	239.46b	173.56	188.89	246.90b	184.47
		RDS	166.17	277.20b	162.10	156.28	283.28b	160.46
		ANOVA	n.s.	*	n.s.	n.s.	*	n.s.

Each value is the mean of the 4 replicates. Different letters on the same parameter and irrigation period (column) denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA rows \*, \*\* refer to significant effect at  $P < 0.05$  or  $0.01$  respectively and n.s. to not significant.

(Table 2). The CTL treatment gave mean values above  $-30$  kPa for all periods during both seasons. Both deficit treatments led to statistical differences with CTL during the 2015 and 2016 postharvest. RDS and RDM reached postharvest mean values below  $200$  and  $-300$  kPa, respectively. The RDM treatment showed the lowest values preharvest, especially during the 2017 preharvest; however, due to its high variability  $\Psi_m$  was not sensitive enough to detect significant differences between treatments during preharvest.

Plant water stress indicators showed values reflected those of  $\Psi_m$ . Tree's MDS resulted in significant differences between CTL and both RD treatments during postharvest, but, as in the case of  $\Psi_m$ , the preharvest stress in RDM was not reflected in the results (Table 2). MDS increased by  $11$  and  $23\%$  in RDM with respect to CTL in 2016 and 2017 pre harvests, respectively.

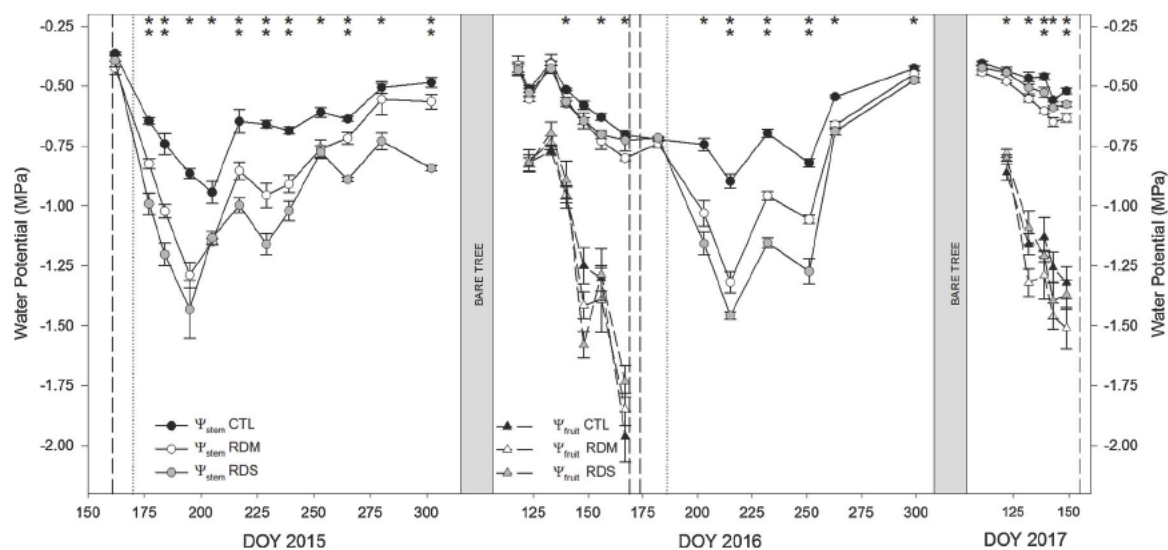
Midday stem water potential reflected differences among treatments, as shown in Fig. 1. During postharvest, there were significant differences between CTL and RDS at every measuring point, while in the measurements corresponding to late July and August,  $\Psi_{stem}$  reflected the differences between RDM and RDS. The minimum  $\Psi_{stem}$  values were reached both seasons by trees of the RDS treatment during postharvest, when they were close to  $1.5$  MPa. During preharvest,  $\Psi_{stem}$  was sensitive enough to identify the mild deficit imposed by RDM, and significant differences between RDM and CTL appeared;

moreover, in 2017 preharvest  $\Psi_{stem}$  was able to distinguish among all treatments. RDM led to  $\Psi_{stem}$  values that were  $0.06$  and  $0.12$  MPa lower than in CTL prior to harvest in 2016 and 2017, respectively (Fig. 1).  $\Psi_{stem}$  was the only water stress indicator that showed significant differences during preharvest.

Fruit water potential did not show significant differences among treatments (Fig. 1) and was strongly related to  $\Psi_{stem}$  (Fig. 2A).  $\Psi_{fruit}$  showed a similar trend to negative values during both preharvest periods; however, in 2016 the mean values in all treatments were  $30\%$  lower than in 2017 (Fig. 1). As was to be expected,  $\Psi_{fruit}$  was seen to be highly dependent on  $\Psi_{nfruit}$  (Fig. 2B), which, in turn, was closely related with the fruit soluble solids content (Fig. 2C). On the other hand,  $\Psi_{pfruit}$  showed a lower linear relationship with  $\Psi_{fruit}$  and explained  $53.6\%$  of the changes in  $\Psi_{fruit}$ . Furthermore, a linear relationship between  $\Psi_{pfruit}$  and fruit growth rate (GR) was obtained ( $R^2 = 0.56$ ; Fig. 2D).

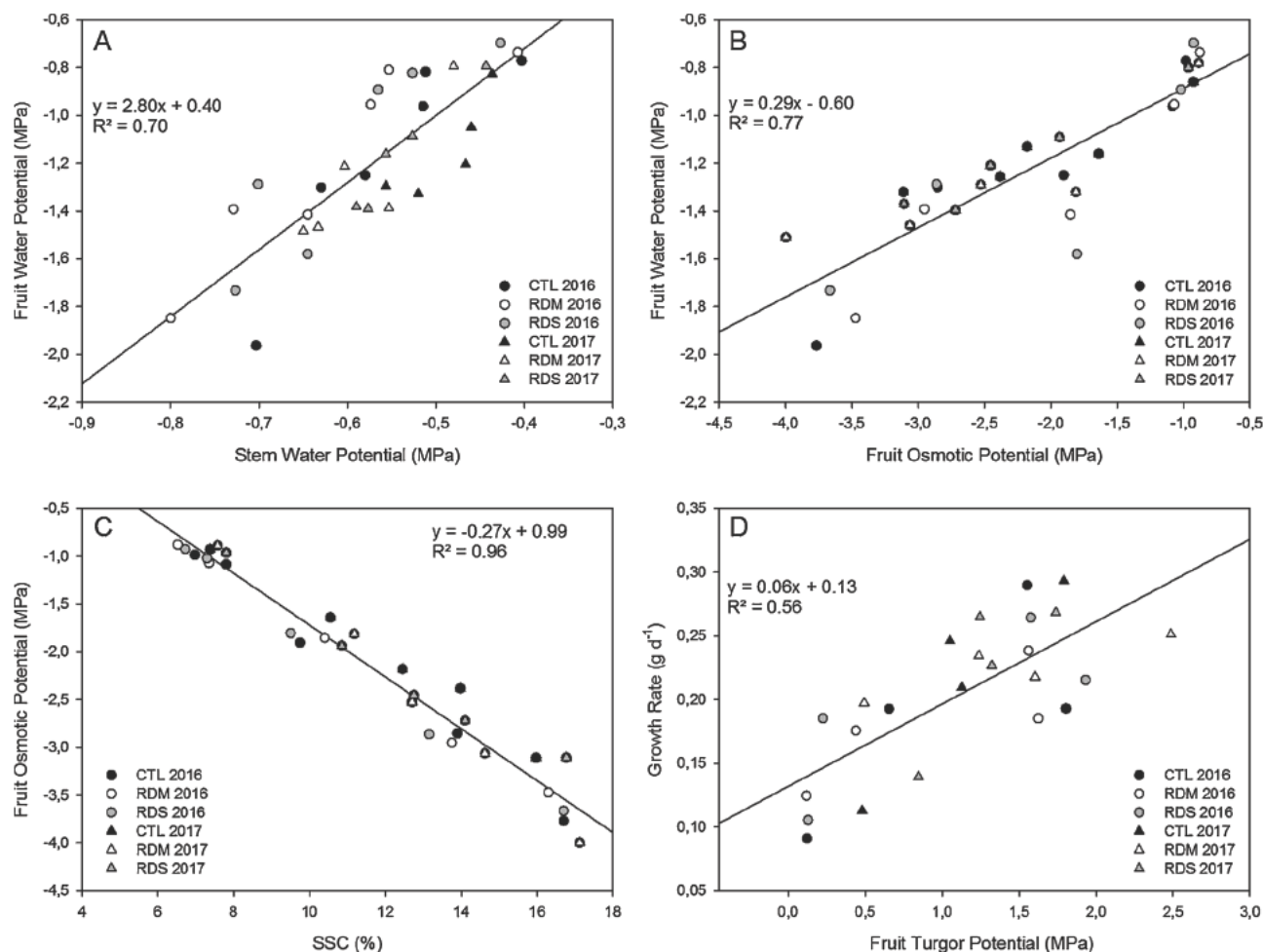
### 3.3. Fruit development dynamic

Fruit development and ripening had finished by  $70$  and  $73$  DAFB in 2016 and 2017 respectively. Fig. 3 shows the increases in fruit size during the fruit development period. The fruit physical parameters volume and fresh unitary weight were characterized by a sigmoidal growth pattern, which could be divided into three distinct parts. The



**Fig. 1.** Evolution of the midday stem water potential ( $\Psi_{stem}$ ) and fruit water potential ( $\Psi_{fruit}$ ) for the irrigation treatments control (CTL), and both regulated deficit irrigation treatments, mild preharvest and medium postharvest deficit irrigation (RDM) and severe postharvest deficit irrigation (RDS).

Each value is the mean of the 6 measures. \* refers to significant differences between CTL and any RD treatment and \*\* refers to significant differences among all treatments according to Duncan multiple range test ( $P < 0.05$ ). Vertical dashed lines show harvest days and dotted line the start of the postharvest deficit period.



**Fig. 2.** Linear relationships between: stem water potential and fruit water potential (A), fruit osmotic potential and fruit water potential (B), soluble solids concentration, SSC, and fruit osmotic potential (C), fruit turgor potential and growth rate (D). Each value is the mean of 3 replicates per treatment.

first part showed an exponential increase up to 18 and 20 DAFB, 117 DOY in 2016 and 100 DOY in 2017, which could be attributed to stage I and II of fruit development. The second part, of similar length, had a linear trend, early mid stage III of fruit development. Fruit growth slowed down in the third part, late stage III, until the final fruit size was reached (commercial harvest). There was no clear evidence of the exponential part in the fruit dry weight and in the equatorial diameter growth pattern compared with fruit volume or SSC. Fruit were larger in 2017 than in 2016, a year in which no treatment produced fruit weighing more than 9 g and measuring more than 8.4 cm<sup>3</sup>, while in 2017 all treatments produced fruit of 11 g and 10.7 cm<sup>3</sup>. In neither year there were differences among treatments as regards fruit size, yield and number of fruit per tree, whereas all these measurements differed between years. Equatorial diameter was the fruit size parameter measured in which the different fruit growth stages were hardest to identify. The fruit fresh weight exhibited a similar trend to that of the fruit volume each year. In 2016, fresh and dry unitary weight showed rapid increase from 30 days before harvest, less pronounced during the last 10 days; in 2017 the fruit and dry unitary weight did not show a slower increase in the previous days to harvest (Fig. 3C and D).

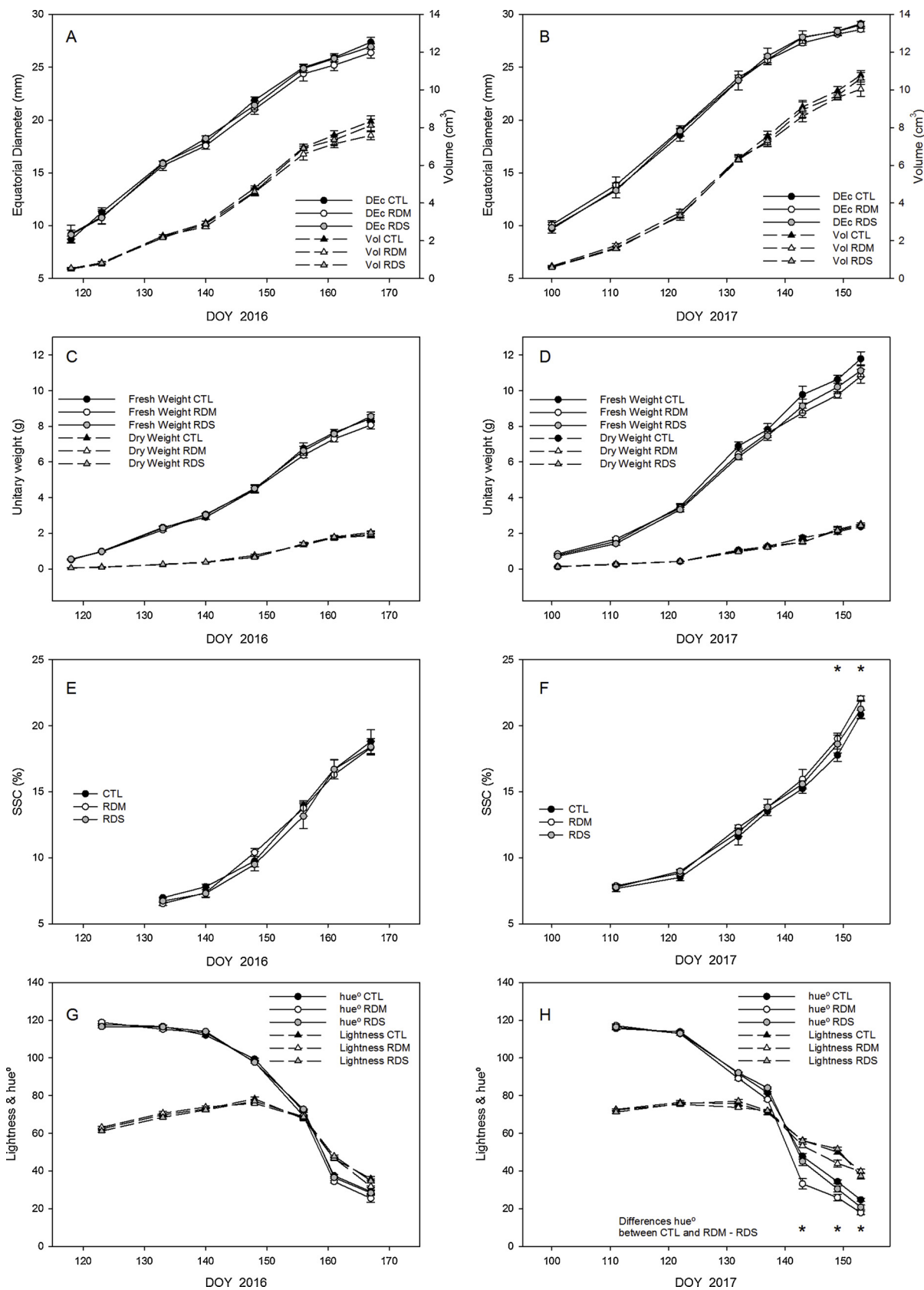
SSC evolution showed a similar pattern to fruit volume and fresh weight (Fig. 3E and F). The greatest SSC increase was seen in all treatments when fruit volume had already reached half of the final size (148 and 132 DOY in 2016 and 2017, respectively). Both years, SSC reached values above 17%; in 2016 SSC values at harvest did not result in significant differences among treatments. However, in 2017, SSC

of fruits from RDM was statistically higher than that from CTL fruits during the last part of the ripening process.

Fruit color changed throughout the ripening stage. The hue<sup>a</sup> showed an inverse sigmoid pattern, with a sharp drop when fruit changed from a green straw colour to red mahogany. This change started approximately 45 DAFB and although the ripening process continued until harvest, to reach a full red color fruit 15 days were necessary both years (Fig. 3G and H). In 2016 neither hue<sup>a</sup> nor L\* was significantly different among treatments. However, in 2017, although there were no significant differences in L\* among treatments, RDM and RDS produced darker cherries compared with CTL fruit (lower values of hue<sup>a</sup>). Once fruit started to change color, RDM led to redder colors than CTL and RDS, but at harvest trees from both deficit irrigation treatments bore darker cherries than CTL.

#### 3.4. Fruit quality and yield

At harvest, the quality parameters analyzed did not show differences among treatments in 2016; however in 2017 RDM fruit were sweeter and darker compared to the fruit from CTL. When the quality parameters of both years were compared, all of them showed significant differences. The fruit from 2016 was more prone to crack, 30% smaller, 40% firmer, less dark red, less sweet and less acid than the fruit from the same irrigation treatments in 2017 (Table 3). These results are



**Fig. 3.** Evolution of fruit equatorial diameter and volume (A and B), fruit unitary fresh and dry weight (C and D), soluble solids concentration, SSC (E and F) and fruit color parameters, lightness and hue° (G and H) during 2016 and 2017 fruit development and ripening of 'Prime Giant' sweet cherries cultivated under three different irrigation treatments: control (CTL) and two deficit irrigation treatments, a mild preharvest and medium postharvest deficit irrigation (RDM) and a severe postharvest deficit irrigation (RDS). Each value is the mean of 4 replicates per treatment. \* refers to significant differences among treatments at each time according to ANOVA ( $P < 0.05$ ).

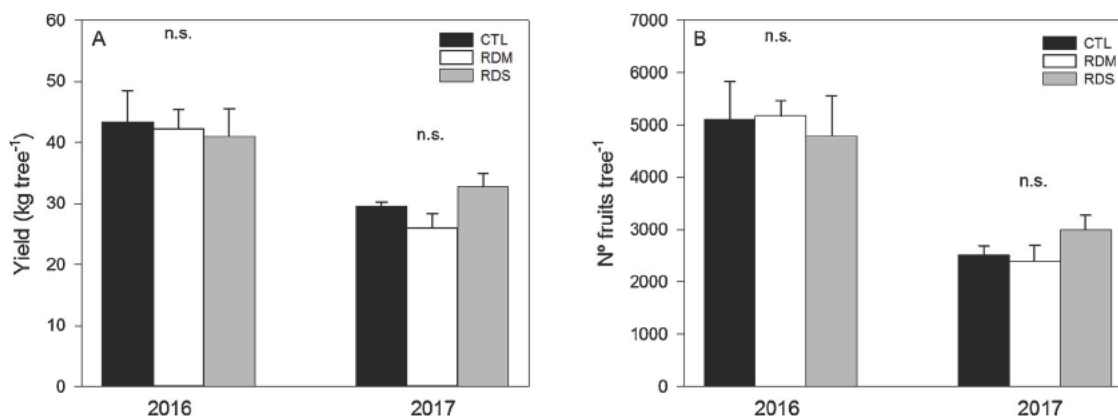
**Table 3**

Mean values for the physicochemical parameters of 'Prime Giant' sweet cherries at harvest observed during the study period (2016–2017) for the irrigation treatments control (CTL), and both regulated deficit irrigation treatments, mild preharvest and medium postharvest deficit irrigation (RDM) and severe postharvest deficit irrigation (RDS).

		Cracking incidence (%)	Equatorial Diameter (mm)	Longitudinal Diameter (mm)	Volume (cm <sup>3</sup> )	Firmness Fruit (N)	Firmness Pulp (N)	Fruit Color (hue <sup>o</sup> )	Pedical Color (hue <sup>o</sup> )	SSC (%)	TA (g 100 ml <sup>-1</sup> )	MI
2016	CTL	9.73	27.36	21.22	8.33	9.23	4.05	25.67	115.36	18.80	1.27	14.80
	RDM	4.58	26.31	20.81	7.54	8.51	4.01	23.90	113.53	18.33	1.16	15.80
	RDS	6.05	26.95	21.33	8.11	8.77	3.75	24.26	113.31	18.40	1.24	14.84
2017	CTL	1.50	29.13	24.29	10.78	6.52	3.66	24.71a	111.73	21.05b	1.41	14.93
	RDM	1.19	28.58	23.47	10.04	6.21	3.37	17.88b	109.97	21.93a	1.43	15.34
	RDS	0.69	29.02	24.13	10.61	6.39	3.38	20.86b	106.78	21.15ab	1.39	15.22
Statistical Analysis												
Treatment (T)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Year (Y)		***	***	***	***	***	***	***	**	**	***	*
T x Y		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	*	n.s.	n.s.

SSC: soluble solids concentration; TA: titratable acidity; MI: maturity index.

Each value is the mean of the 4 replicates. Different letters on the same parameter and year (column) denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ). In the statistical analysis \*, \*\* and \*\*\* refer to significant effect at  $P < 0.05$ , 0.01 and 0.001 respectively and n.s. to not significant.



**Fig. 4.** Influence of irrigation treatment on yield (A) and number of fruits per tree (B) over the seasons (2016–2017) of 'Prime Giant' sweet cherries cultivated under three different irrigation treatments: control (CTL) and two deficit irrigation treatments, a mild preharvest and medium postharvest deficit irrigation (RDM) and a severe postharvest deficit irrigation (RDS).

Each value is the mean of the 4 replicates. n.s. denotes no significant differences among treatments according to ANOVA ( $P < 0.05$ ).

consistent with the yield measured each year, 2016 was a high cropping year and trees produced 43% more kg per tree (42 vs. 29 kg fruit tree<sup>-1</sup>) and a 91% more fruits per tree (5027 vs. 2634) than 2017 (Fig. 4); consequently fruits from 2016 were significantly smaller. Furthermore, when the interaction of both, irrigation treatment and year conditions was considered, parameters such as fruit color and SSC showed a significant interaction (Table 3).

### 3.5. Cold storage and shelf life extension performance

During cold storage, the physical parameters related with fruit size equatorial and polar diameters and volume remained stable throughout the experiment (Table 4). Fruit and pulp firmness increased significantly with time in cold storage for all treatments and sharply declined during the shelf life simulation. Although there were no statistical differences among treatments, CTL produced firmer fruit than the deficit treatments, especially pulp firmness (Fig. 5).

At the 2016 harvest, sweet cherry showed hue<sup>o</sup> values close to 24 for all treatments, which is associated with bright red colors. However, during cold storage and during shelf life simulation a reduction in this parameter was observed particularly in the fruits from CTL. Similarly,

pedicel color also decreased as time passed. At harvest, all pedicels had a bright green color (up to 110 hue<sup>o</sup>) regardless of the irrigation treatment applied; however after cold storage, the pedicels from CTL fruit were significantly more greenish to straw colored than those from RDS and RDM which remained bright green. During shelf life simulation the pedicels from CTL turned a light brownish color, while the others maintained greener colors, especially RDM, but, due to high variability within each treatment, no significant differences were observed (Fig. 5).

Regarding SSC and TA, there were no significant differences among treatments. However, both parameters tended to slightly lower mean values than those measured at harvest (Fig. 5). SSC and TA values after the experiment decreased 5 and 9%, respectively compared to the values measured at harvest. As a result of the greater decrease of TA than SSC, the MI increased. RDM led to higher values of MI, with no significant differences among treatments.

## 4. Discussion

The results showed that  $\Psi_m$ , as well as plant water stress indicators such as MDS and  $\Psi_{stem}$ , in CTL showed the typical water status of well

**Table 4**

Mean values for the physicochemical parameters of sweet cherries at 2016 harvest (t0), after 20 days of cold storage at 2 °C (t1) and after 5 days of self-life at 15 °C (t2) for the irrigation treatments control (CTL), and both regulated deficit irrigation treatments, mild preharvest and medium postharvest deficit irrigation (RDM) and severe postharvest deficit irrigation (RDS).

		Equatorial Diameter	Longitudinal Diameter	Volume	Firmness Fruit	Firmness Pulp	Fruit Color	Pedice Color	SSC	TA	MI
		(mm)	(mm)	(cm <sup>3</sup> )	(N)	(N)	(hue <sup>o</sup> )	(hue <sup>o</sup> )	(%)	(g 100 ml <sup>-1</sup> )	
Treatment (T)	CTL	27.29	21.33	8.33	9.36	3.96	24.26	101.83	18.51	1.23	15.10
	RDM	26.39	21.00	7.66	8.72	3.74	23.19	107.36	18.09	1.16	15.71
	RDS	27.05	21.47	8.23	9.21	3.65	23.99	104.30	18.27	1.21	15.16
Time (t)	t0	26.87	21.12	7.99	8.83b	3.93b	24.61a	114.07a	18.51	1.26	14.72
	t1	27.03	21.31	8.16	10.37a	4.39a	24.06a	107.14b	18.29	1.20	15.29
	t2	26.83	21.38	8.07	8.09b	3.03c	22.77b	92.28c	18.07	1.14	15.96
Statistical Analysis											
Treatment (T)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Time (t)		n.s.	n.s.	n.s.	***	***	**	***	n.s.	n.s.	n.s.
T x t		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

SSC: soluble solids concentration; TA: titratable acidity; MI: maturity index.

Each value is the mean of the 3 replicates. Different letters on the same parameter denote significant differences among treatments or times according to Duncan multiple range test ( $P < 0.05$ ). In the statistical analysis \*\* and \*\*\* refer to significant effect at  $P < 0.01$  or  $0.001$  respectively and n.s. to not significant.

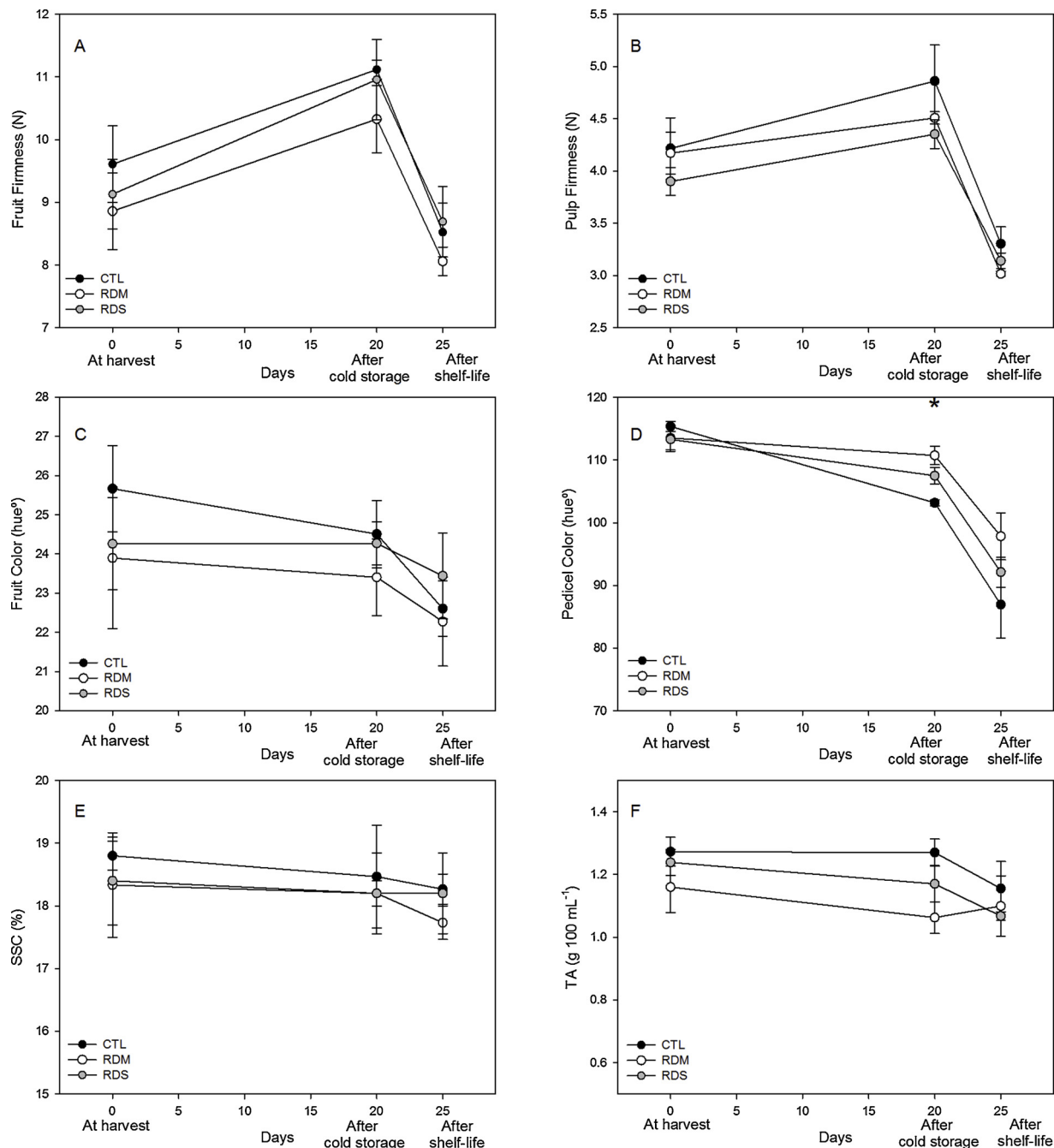
watered trees throughout the experiment. The differences among treatments in all the water indicators during postharvest reflected the irrigation changes in the deficit treatments (Table 2). Since the plant response to soil water deficit depended on the time, duration and intensity of water stress, it is supposed that the soil water deficit reached was enough to induce plant water stress, particularly under drip irrigation conditions. Furthermore, sweet cherry has been described as a highly sensitive crop to soil water deficits and water evaporative demand (Molina et al., 2016). During preharvest, MDS in RDM trees was higher than in CTL in 2016 and 2017, although as the variability among trees was high, these increases in branch fluctuations were not sufficient to lead to statistical differences (Table 2). However, when the water reductions applied in postharvest were high (irrigation amount equivalent to 65 and 55% of  $ET_c$  in RDM and RDS, respectively) the MDS of the trees sharply increased and significant differences appeared, making MDS a reliable indicator of tree water status (Abdelfatah et al., 2013), which could be of interest for irrigation scheduling in sweet cherry trees. Although all indicators were able to identify a water deficit situation, only  $\Psi_{stem}$  in trees from RDM identified mild water stress and provided significant differences during preharvest (Fig. 1). Thus, our results confirm  $\Psi_{stem}$  as a reference indicator in deficit irrigation management in sweet cherry, as previously reported by Blanco et al. (2018); Marsal et al. (2009) and Oyarzun et al. (2010).

$\Psi_{fruit}$  showed a high linear relationship with  $\Psi_{stem}$  (Fig. 2A), although slightly lower than that reported in peach by Gelly et al. (2004).  $\Psi_{fruit}$  showed a similar trend to negative values as  $\Psi_{stem}$  (Fig. 1). However,  $\Psi_{fruit}$  tended to decrease more sharply as the fruit developed regardless of irrigation treatment; similar results have been described in different crops such as apple (Mills et al., 1997), jujube (Galindo et al., 2016), olive (Fernandes et al., 2018), pomegranate (Galindo et al., 2017), and others. A substantial decrease in  $\Psi_{fruit}$  was observed 27 and 31 days before fruit harvest in 2016 and 2017 that (0.46 and 0.44 MPa in 2016 and 2017, respectively) (Fig. 1). This decrease coincided with the early stage III of fruit development, the phase with the highest growth, SSC increase and fruit color change (Fig. 3). The high linear relationship between  $\Psi_{stem}$  and  $\Psi_{fruit}$ , especially during stage III of sweet cherry development, is consistent with the remarks of Bertin and Génard (2018), who reported that cell enlargement is a result of water flows following a stem to fruit gradient of water potential. The recorded decreases in  $\Psi_{fruit}$  coincided with the decrease in  $\Psi_{nfruit}$  and the increases in fruit size and SSC. This increase in SSC was strongly related to  $\Psi_{nfruit}$  decreases (Fig. 2C), this linear relationship obtained between SSC and  $\Psi_{nfruit}$  being similar to the one reported by Yakushiji et al. (1996) in satsuma mandarin. These observations were also consistent

with the  $\Psi_{nfruit}$  and  $\Psi_{fruit}$  evolution described by Schumann et al. (2014) in sweet cherry 'Regina'. Therefore, a high linear relationship between  $\Psi_{nfruit}$  and  $\Psi_{fruit}$  was obtained (Fig. 2B). The rapid and high decreases in  $\Psi_{nfruit}$  were balanced by corresponding increases in  $\Psi_{pfruit}$ .  $\Psi_{pfruit}$  values were always maintained above zero in all treatments throughout the experiment (Fig. 2D). The maintenance of  $\Psi_{pfruit}$  values above zero is fundamental during fruit growth and it is known that fruit growth (as cell growth) requires cell turgor (Matthews and Shackel, 2005). Thus, a linear relationship between sweet cherry growth rate and  $\Psi_{pfruit}$  was found. Moreover, in apple, Archbold (1992) stated that carbohydrates partition between the starch and fruit soluble sugars maintained fruit turgor so fruit growth rate (from an osmoregulatory point of view) also depends on  $\Psi_{nfruit}$ . From this relationship it was hypothesized that although  $\Psi_{pfruit}$  remained positive throughout fruit development, factors other than turgor would also have determined the fruit growth rate. Fruit absolute growth increased continuously throughout fruit development (Fig. 3A and B) as did  $\Psi_{pfruit}$ . On the other hand,  $\Psi_{fruit}$  and  $\Psi_{nfruit}$  rapidly decreased as SSC rose.

Matthews and Shackel (2005) described that fruit growth is highly dependent on changes in the structure of the cell wall and the variations in cell turgor and  $\Psi_{nfruit}$ . According to Andrews and Li (1995) the changes in the cell walls (weakening) during sweet cherry ripening and the accumulation of biochemical substance in the fruit lower  $\Psi_{nfruit}$  and play a major role in promoting fruit growth. Thus, sweet cherry growth was conditioned by an increase in  $\Psi_{pfruit}$  values caused by solute accumulation and the cell wall weakening (which diminished  $\Psi_{nfruit}$ ). Thus, source and sink  $\Psi_{nfruit}$  gradients increase  $\Psi_{pfruit}$  values due to water entering through the fruit phloem, which has a significant effect on fruit growth and  $\Psi_{fruit}$  (Bertin and Génard, 2018). Consequently, when the relationship between  $\Psi_{fruit}$  and its two components were compared,  $\Psi_{fruit}$  was seen to be much more influenced by  $\Psi_{nfruit}$  than by  $\Psi_{pfruit}$ .

As  $\Psi_{fruit}$  and  $\Psi_{nfruit}$  declined, so fruit size, weight and SSC increased. As 'Prime Giant' is an early cultivar, growth dynamics did not display the typical double sigmoid pattern of the stone fruits described by Coombe (1976) in which three developmental stages are clearly identified; however, different fruit growth rates could be distinguished (Fig. 3A and B). Our results were consistent with those of Grossman and DeJong (1995) and Papenfuss and Black (2010), who stated that in early cultivars of peach and tart cherry, stage II of fruit development, pit hardening, overlaps stages I and III, cell division and cell enlargement, respectively. The greatest increase in fresh fruit unitary weight, the initial rise in dry fruit unitary weight (Fig. 3C and D) and increase in SSC (Fig. 3G and H) coincided with the beginning of stage III



**Fig. 5.** Mean values of the physicochemical parameters at 2016 harvest, after 20 days of cold storage at 2 °C and after 5 days of shelf-life at 15 °C of sweet cherries ‘Prime Giant’ under three different irrigation treatments: control (CTL) and two deficit irrigation treatments, a mild preharvest and medium postharvest deficit irrigation (RDM) and a severe postharvest deficit irrigation (RDS).

SSC: soluble solids concentration; TA: titratable acidity.

Each value is the mean of 3 replicates per treatment. \* refers to significant differences among treatments at each time according to ANOVA ( $P < 0.05$ ).

(approximately 20 days before harvest), when the rapid decrease of  $\Psi_{\text{fruit}}$  occurred (Knoche et al., 2001). During these days, the growth rate of both years reached their respective maxima (Fig. 3C and D), which coincided with the highest increases of  $\Psi_{\text{fruit}}$ . Furthermore, during these days fruit started their color change from green to straw (Fig. 3G and H). In 2017 RDM led to significantly darker mahogany colors than CTL. Darker colors are associated by the consumer with sweeter fruit so darker fruit are more appreciated (Crisosto et al., 2003). In this study, these results were corroborated. Thus, the darker fruit from RDM were also significantly sweeter than those from CTL. At harvest, fruit quality was evaluated every year. In 2016, the different irrigation

strategies did not prompt significant differences in the quality parameters measured. However in 2017, differences among treatments appeared. RDM produced significantly darker and sweeter fruit (Table 3). Deficit irrigation has been used to improve fruit color in several fruit crops such as peach (Sotiropoulos et al., 2010; Falagán et al., 2015b), nectarine (Falagán et al., 2015a), pomegranate (Peña et al., 2013), table grapes (Conesa et al., 2015, 2016), it being hypothesized that deficit irrigation improves fruit coloration by increasing accumulation of the bioactive compounds involved in the fruit ripening process and diminishes vegetative growth, thus allowing fruit to be more exposed to sunlight. The results for other parameters, such as cracking incidence,

equatorial and polar diameters, volume, firmness, pedicel color, TA and fruit dry unitary weight, were not statistically influenced by the irrigation management, which is consistent with the data reported in ‘Summit’ sweet cherries under RDI strategies by Marsal et al. (2010).

The quality parameters studied were significantly different each year (Table 3). In general, fruit harvested in 2016 were smaller in all treatments than the values expected for this cultivar, while the mean values obtained in 2017 were similar in color, size and SSC and slightly higher for fruit firmness and TA than those reported in the same cultivar by Serrano et al. (2009).

These differences between years could be explained by the differences in yield and number of fruits produced each year. The yield and the number of fruits per tree were 43 and 91% higher in 2016 than in 2017 without differences among treatments within the same year (Fig. 4). Ayala and Lang (2004) and Neilsen et al. (2007) stated that in sweet cherry, in high cropping years such as 2016, quality parameters and especially fruit size are influenced by a low leaf area/fruit ratio and consequently mean fruit unitary weight is lower than in those years with normal yield and higher leaf area/fruit ratio. Olmstead et al. (2007) reported that sweet cherry size was more influenced by cell length than by number of cells, so consequently environmental conditions and sink limitations affected more final cherry size during stage III of fruit development (cell enlargement) than during stage I (cell division). Smaller fruit in 2016 induced higher firmness, brighter red color and lower concentration of TA. When the physicochemical parameters of sweet cherries at harvest for both years were compared, the interaction treatment year was only significant for hue<sup>o</sup> and SSC confirming that these are the parameters more sensitive to irrigation management.

Irrigation treatments did not affect cracking incidence, nor did it penalize fruit yield and number of fruits per tree (Table 3, Fig. 4A and B). However, although RDM did not show significant differences in fruit unitary weight compared to CTL, CTL had larger fruits both years, in 2017 fruit unitary weight was almost 1 g higher in the fruits produced by CTL than those of RDM (Fig. 3C and D). Lower mean unitary weights in deficit treatments, although without significant differences, have been reported by Goldhamer et al. (1999) in peach. It is well known that the crop profitability of sweet cherry is highly dependent on commercial size categories (Nieto et al., 2017), so the decreases obtained by RDM treatment could have a negative impact on economic returns. Other studies on regulated deficit irrigation related the stress integral (Myers, 1988) with the number of fruits per tree. However, the present study showed low correlation between stress integral and number of fruits per tree when both years are considered, due to the similar amount of water applied to each specific treatment each year (Table 1) and the different yield obtained (Fig. 4A). Thus, the low correlation between those stress integral and number of fruits per tree when both years are considered could be due to other factors, and/or a combined effect of the same: such as the preharvest mild water stress on RDM trees, as soil water deficits have been related to smaller fruit in sweet cherry (Neilsen et al., 2014), a postharvest deficit that could provoke effects on the fruit quality of the following season, temperature and evaporative demand during bloom, fruit set and cherry run off, different crop loads, vegetative growth, sink source relations (Ayala and Lang, 2015).

Fruit quality from the first pick of 2016 was evaluated at harvest, after cold storage (20 days at 2 °C) and a subsequent shelf life simulation period (5 days at 15 °C) to assess the effect of deficit irrigation on the storage performance of sweet cherry. Size parameters seemed to remain stable during storage in all treatments (Table 4). Fruit and pulp firmness after cold storage increased as an effect of low temperatures during storage, similar to the results obtained in ‘Bing’ cherries after 6 weeks at 1.67 °C by Patterson and Kupferman (1983). However, fruit and pulp firmness dropped during commercial life conditions (Table 4 and Fig. 5A and B). Fruit color remained almost constant throughout the cold storage but it turned into darker colors during shelf life (Fig. 5C). The greatest changes were to pedicel color, the only factor

that was influenced by deficit irrigation. RDM led to a greener color at the end of the cold storage than CTL. CTL pedicels showed significantly more discoloration (browning) after 20 days at 2 °C. However, at the end of the experiment, after 5 days at shelf life conditions (15 °C) the color of the pedicels from all treatments was degraded. Pedicel shriveling in sweet cherry is highly dependent on temperature and relative humidity (Knoche et al., 2015) and although the pedicels from the fruit of the RDM treatment were greener than those from CTL, differences among treatments were not detectable at the end of the experiment (Fig. 5D). Sweet cherry pedicel has been described as one of the best indicators of its freshness, and greener pedicels are related to higher consumer acceptance (Knoche et al., 2015). The greener colour of the pedicel from the deficit treatments was related with lower dehydration, as lower dehydration losses in fruits from trees under regulated deficit irrigation have been explained as a result of the thicker cuticle in these fruits, which reduces water losses (Gómez del Campo et al., 2014; Pérez Pastor et al., 2007; Romero Trigueros et al., 2017). SSC and TA did not show differences among irrigation treatments or times (at harvest, after cold storage or after shelf life) (Table 4), with mean values above 18% and 1.1 g 100 ml<sup>-1</sup> in all measurements. According to the results obtained, deficit irrigation treatments did not penalize any of the physicochemical quality parameters studied. Nevertheless, as could be expected, parameters such as fruit and pulp firmness and skin and pedicel color presented significant differences between the values obtained at harvest and after cold storage and shelf life (Table 4). This is indicative of the loss in sweet cherry fruit quality as storage progresses, although no interaction with the irrigation treatment was observed. Sweet cherry has been described as a highly perishable fruit which deteriorates rapidly after harvest. However, in our experiment, the mean values of the quality parameters obtained after 20 days at 2 °C and 5 days at 15 °C, such as mahogany fruit color (23 hue<sup>o</sup>), green pedicels (92 hue<sup>o</sup>), good firmness (8 N), SSC values above 15% and TA between 0.4 and 1.5 g 100 ml<sup>-1</sup>, can be considered acceptable in the fruit from all the irrigation treatments (Habib et al., 2015). ‘Prime Giant’ sweet cherry then, can be safely marketed after cold storage as the fruit will still be found acceptable by consumers.

## 5. Conclusion

The application of RDM and RDS produced water savings of 36 and 40% of the water applied in the CTL treatment without significantly penalizing fruit yield or quality.  $\Psi_{stem}$  was the water deficit indicator that best identified and quantified the plant water status in sweet cherry. The biggest changes in  $\Psi_{fruit}$  took place in the period from the onset of rapid cherry growth (first part of stage III) which was accompanied by a rapid increase in the fruit turgor potential and a decrease in fruit osmotic potential. These results indicate the need to maintain a positive pressure potential inside the cell and a continuous supply of biochemical substances for the proper growth of the fruit.

Regulated deficit irrigation treatments did not produce differences in the quality parameters measured at the 2016 harvest compared with CTL. However, after 20 days of cold storage, RD led to greener pedicels than CTL. This difference among treatments could be a key factor in leading consumers to prefer RD sweet cherries over those from CTL due to their visual appearance after storage. In the 2017 harvest, RD, particularly RDM, led to darker and sweeter fruit than CTL. When both deficit treatments were compared, RDM did not improve RDS fruit quality. Furthermore, fruits from RDS did not show any size reduction compared to CTL either year of study. Based on these results, RDS can be recommended over the other irrigation strategies to improve water use in semiarid areas without negatively affecting yield or fruit size, while even enhancing some quality parameters.

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Article III

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# Vegetative and reproductive response of ‘Prime Giant’ sweet cherry trees to regulated deficit irrigation

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## ABSTRACT

The agronomic response of sweet cherry (*Prunus avium* L.) ‘Prime Giant’ to a 4 year-long experiment involving deficit irrigation strategies in a Mediterranean climate was studied in a commercial orchard located in the southeast of Spain (Jumilla, Spain). Four drip irrigation treatments were imposed: (i) control treatment (CTL), irrigated without restrictions at 110% of seasonal crop evapotranspiration (ET<sub>c</sub>); (ii) sustained deficit irrigation (SDI) treatment irrigated at 85% ET<sub>c</sub> during pre-harvest and post-harvest periods and at 100% ET<sub>c</sub> during floral differentiation; (iii) regulated deficit irrigation (RDI) treatment irrigated at 100% ET<sub>c</sub> during pre-harvest and floral differentiation and at 55% ET<sub>c</sub> during post-harvest, and (iv) farmer treatment (FRM), irrigated according to the farmer’s normal practice. The crop’s response to the different irrigation treatments was analyzed in relation to tree water status. Soil water deficit reduced tree midday stem water potential ( $\Psi_{stem}$ ), stomatal conductance (gs) and net photosynthesis (Pn). Branch maximum daily shrinkage (MDS) responded rapidly to irrigation changes during pre-harvest and post-harvest. The lowest  $\Psi_{stem}$  values were reached by SDI during pre-harvest and by RDI and FRM during post-harvest. RDI did not lead to  $\Psi_{stem}$  water potentials falling to below the threshold of  $-1.6$  MPa in any season, although, FRM caused,  $\Psi_{stem}$  to fall below  $-1.8$  MPa in 2017.

RDI reduced vegetative growth and did not cause significant lower yields or fruit quality. However, with SDI there was a trend towards smaller fruits and a slightly higher soluble solid content. Post-harvest deficit irrigation increased water productivity without penalizing fruit yield or the quality parameters studied, and allowed water savings of 39% compared to CTL at a time when other fruit tree species require more water. Moreover, RDI and SDI led to significantly less cracking incidence and a lower cracking index, which could extend fruit shelf life.

## 1. Introduction

Irrigated agriculture has long been and will continue to be the main consumer of water worldwide (UNESCO, 2001). Indeed, 40% of the total world food supply currently depends on the irrigated agriculture, while occupying only 17% of the world’s agricultural land (FAO, 2002). Water is a scarce resource and the development of industry and cities requires increasingly large amounts of fresh water. Thus, there is a constant pressure on irrigated agriculture to conserve water, land and

energy, while increasing food and fibre production (Feres and Evans, 2006). Moreover, agriculture should be prepared to face new challenges such as climate change, which is already modifying water availability worldwide. Irrigation strategies that conserve water resources will be part of the solution to ensure the production of safe food and protection of the environment. Therefore, irrigation management needs to be optimized to increase water use efficiency in agriculture, avoiding the unnecessary waste of this important and limited resource (Saccon, 2018).

**Abbreviations:** BGR, daily branch growth rate; CTL, control treatment-full irrigation; ET<sub>0</sub>, reference crop evapotranspiration; ET<sub>c</sub>, crop evapotranspiration; FE, fruit efficiency; FRM, farmer treatment; FTI, fruit number per trunk increment; gs, stomatal conductance; IWUE, intrinsic water use efficiency; Kc, crop coefficient; Kr, localization coefficient; LVDT, linear variable differential transformer; MDS, maximum daily branch shrinkage; PFR, photosynthetic photon flux rate; Pn, net photosynthesis; RDI, regulated deficit irrigation; SDI, sustained deficit irrigation; SI<sub>MDS</sub>, maximum daily branch shrinkage signal intensity; SSC, soluble solids concentration; TCSA, trunk cross-sectional area; VPD, vapour pressure deficit; WP, water productivity; YE, yield efficiency  $\theta_v$  soil volumetric water content referred to field capacity;  $\Psi_m$ , soil matric potential;  $\Psi_{stem}$ , midday stem water potential

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One of the most promising avenues for improving water productivity in certain plant species is the use of regulated deficit irrigation (RDI) strategies. Chalmers et al. (1981) defined regulated deficit irrigation as a strategy which consists of reducing water supplied to the crop during specific phenological stages to manage crop vegetative growth and improve water efficiency without penalizing fruit yield or quality.

RDI effects on tree crops such as nectarine, peach and apricot have been studied, and the results indicate that yield, fruit size and fruit quality can be maintained with water savings of around 40% (de la Rosa et al., 2015; Girona et al., 2005; Pérez-Pastor et al., 2009; Torrecillas et al., 2000). However, when drought stress is excessive or is applied at the wrong moment, both yield and fruit size are affected as has been reported in plum and apricot (Intrigliolo and Castel, 2006; Torrecillas et al., 2000). There is limited information on the response of sweet cherry to drought and deficit irrigation strategies (Dehghanisanij et al., 2007; Livellara et al., 2011; Marsal et al., 2009, 2010; Nieto et al., 2017). Moreover, there is an even greater scarcity of information about the effects of RDI on long term yield, fruit quality and vegetative growth of sweet cherry in Mediterranean conditions.

RDI in stone fruits usually involves applying deficit irrigation during stage II of fruit development (pit hardening). However, in sweet cherry and in early cultivars of prune trees such as 'Flanoba' nectarine, whose fruit develops rapidly, stage II is indistinguishable and overlaps stage I and III. For this reason, it is not recommendable to apply water deficit at any stage of fruit growth in early and extra early cultivars including sweet cherry trees (de la Rosa et al., 2015; Marsal, 2012).

Sweet cherry (*Prunus avium* L.), a non-climacteric stone fruit of the genus *Prunus*, is held in high regard by consumers, due to its organoleptic and nutritional characteristics, and by growers because of the good returns it provides. Worldwide production of fresh cherries has increased by 35% in the last 20 years, reaching 2.2 Mt (Tricase et al., 2017) with Spain the 5<sup>th</sup> greatest producer (FAOSTAT - Food and Agriculture Organization of the United Nations Statistics Division, 2015). Sweet cherry has been described as being highly sensitive to water deficit during pre-harvest. Nevertheless, despite the frequency of summer droughts in the Mediterranean Basin, there is a lack of information on the effect of deficit irrigation on the physiological and agronomical response of sweet cherry trees (Centritto, 2005).

The objective of this work was to study the effects of different deficit irrigation strategies on the water status, yield and vegetative growth of adult 'Prime Giant' sweet cherry trees in order to optimise irrigation water management in a semiarid area with scarce water resources.

## 2. Materials and methods

### 2.1. Site description

The experiment was conducted at a 0.5 ha commercial orchard located in Jumilla (Murcia, Spain, 38° 8' N; 1° 22' W, altitude 670 m) from 2015 to 2018. The area has a typical semi-arid Mediterranean climate characterized by wet mild winters and hot dry summers. The soil is moderately stony, the texture sandy loam with a particle size distribution of 67.5% sand, 17.5% silt and 15% clay, with a high level of assimilable phosphorus (108.67 mg kg<sup>-1</sup>) and an adequate exchangeable potassium (3.2 mmol kg<sup>-1</sup>) content. The irrigation water, which comes from a well, had an average electrical conductivity (EC<sub>25°C</sub>) of 0.8 dS m<sup>-1</sup>, with maximum concentration of sodium and chloride of 1.7 and 1.05 mmol L<sup>-1</sup>, respectively.

### 2.2. Experimental design and treatments

The study was carried out in fifteen year-old mature sweet cherry trees (*P. avium* L. 'Prime Giant') grafted on SL64 rootstock and 'Early Lory' and 'Brooks' as pollenizers, at a plant density of 667 trees ha<sup>-1</sup>. Drip irrigation consisted of a single drip line per tree row and three

pressure-compensated emitters per tree of 4 L h<sup>-1</sup> each. Fertilization was the same for all treatments and regardless of the water applied. The fertilization programme applied consisted of 63, 30, 107 and 8 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and CaO, respectively, in the drip irrigation water with the aim of re-establishing the levels of nutrients taken up by mature sweet cherry trees. Fertilization, pruning, weed and pest control were the same for all trees and were consistent with local management practices. The irrigation was applied during the dry period, from March before flowering until November. Full bloom was in April, and annual pruning was carried out in August (approximately 60 d after harvesting).

The experiment involved four irrigation treatments: (i) a control treatment (CTL) irrigated to satisfy maximum crop evapotranspiration (ET<sub>c</sub>) throughout the growing season (110% ET<sub>c</sub>); (ii) a sustained deficit irrigation treatment (SDI), irrigated at 85% of ET<sub>c</sub> during pre-harvest and post-harvest except for the 15–20 days after the first harvest (floral differentiation), when trees were irrigated at 100% ET<sub>c</sub>; (iii) a regulated deficit irrigation treatment (RDI), irrigated at 100% of ET<sub>c</sub> during pre-harvest and the first 15–20 days of flower differentiation and 55% of ET<sub>c</sub> during post-harvest (a non-critical period), and (iv) farmer treatment (FRM), irrigated according to the farmer's normal practice which consists of irrigating above the crop water requirements during pre-harvest and applying uncontrolled water deficit during post-harvest.

Crop evapotranspiration under drip irrigation (ET<sub>c</sub>) was estimated using the equation: ET<sub>c</sub> = ET<sub>o</sub> × K<sub>c</sub> × K<sub>r</sub>, where ET<sub>o</sub> is the average value of the evapotranspiration during the 3–5 days prior to applying the new irrigation scheduling, K<sub>c</sub> is a crop-specific coefficient based on Marsal (2012), which varies from 0.3 in March and November to 0.96 in June and July, and K<sub>r</sub> is a localization factor based on Fereres et al. (1982) and related to the percentage of ground covered by the crop (K<sub>r</sub> = 0.90).

### 2.3. Meteorological conditions

Daily climatic data such as air temperature, air relative humidity, rainfall and crop reference evapotranspiration were recorded by an automatic weather station near the experimental orchard owned by the Spanish agroclimatic information service (SIAR; <http://crea.uclm.es/siar/datmeteo/>). From the temperature and humidity data, the vapour pressure deficit (VPD) was calculated according to Allen et al. (1998).

### 2.4. Soil water status

Soil volumetric water content, was obtained with two FDR sensors (Enviroscan, Sentek Pty. Ltd., Adelaide, Australia) per replicate at 20 and 40 cm depth located 0.23 m from the emitter and 1.5 m from the trunk of the central tree of each block, under the canopy shade. The daily minimum value of the soil volumetric water content is referenced to field capacity as a percentage of the maximum soil water content available in the soil (θ<sub>v</sub>, %). Likewise, the matric potential of the soil (Ψ<sub>m</sub>, kPa) was measured in one tree per replicate by means of two thermal compensation capacitive sensors (MPS-6, Decagon Devices Inc., Pullman, WA, USA) at 25 and 50 cm depth and at a distance of 0.23 m from the emitter. The mean value of Ψ<sub>m</sub> from 11:00 to 14:00 h (solar time) was calculated.

### 2.5. Plant water status

The plant water status was monitored approximately every ten days by measuring stem water potential at noon (Ψ<sub>stem</sub>; MPa) with a Scholander pressure chamber (Model 3000, Soil Moisture Equipment, Santa Barbara, CA, USA), according to the methodology proposed by McCutchan and Shackel (1992) in 6 trees per treatment equipped with linear variable differential transformer (LVDT) sensors. The mature and healthy leaves selected were from the north quadrant close to the trunk,

thus avoiding solar exposure. Leaves were enclosed in black polyethylene bags and covered with aluminium foil at least 2 h before measurement. Likewise and also at noon, gas exchange measurements were measured in four sun-exposed leaves of the outer canopy per replicate. Maximum stomatal conductance ( $g_s$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and net photosynthesis ( $P_n$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were measured at a photosynthetic photon flux density of  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , near constant ambient  $\text{CO}_2$  concentration ( $\approx 380 \mu\text{mol mol}^{-1}$ ) and leaf temperature ( $\approx 25^\circ\text{C}$ ) using a portable gas exchange system CIRAS<sup>2</sup> (PP Systems, Hitchin, Hertfordshire, UK). Intrinsic water use efficiency (IWUE) was calculated as the ratio between  $P_n$  and  $g_s$  ( $\mu\text{mol mol}^{-1}$ ).

Branch diameter fluctuations were recorded by two dendrometers (LVDT sensors, model DF  $\pm 2.5$  mm, accuracy  $\pm 10 \mu\text{m}$ , Solartron Metrology, Bognor Regis, UK) per replicate, each placed on a main tree branch away from direct sunlight. The sensors were installed on aluminium and invar holders to prevent thermal expansion. LVDT measurements were performed in differential input configurations. The maximum daily branch shrinkage (MDS) was calculated as the daily difference in diameter between the maximum and the minimum. MDS signal intensity ( $SI_{\text{MDS}}$ ) was calculated by taking the ratio of each treatment versus CTL ( $SI_{\text{MDS}} = \text{MDS}_{\text{TREATMENT}} / \text{MDS}_{\text{CTL}}$ ).  $SI_{\text{MDS}}$  is a dimensionless variable, where one (unity) is equivalent to the absence of water deficit and values above one indicate plant water deficit (Goldhamer and Fereres, 2001). Daily branch growth rate (BGR) was calculated as the difference in diameter between the maximum of two consecutive days (Goldhamer and Fereres, 2001).

Branch diameter fluctuations, matric potential and volumetric water content measurements were recorded every 30 s and the datalogger was programmed to report means every 10 min. Two replicates per treatment were equipped with a wired platform of one datalogger and two multiplexers (Campbell Scientific, Logan, UT, USA) while the other two replicates used a ZigBee wireless sensor network (Widhoc Smart Solutions SL, Fuente Alamo, Murcia, Spain) configured in a star topology (Morais et al., 2008). Data access was by WIFI radio-link provided by a local internet supplier.

## 2.6. Vegetative growth

Vegetative growth was measured as pruning wood, canopy volume, shaded area, cumulate shoot growth and trunk cross-sectional area. The pruning wood was expressed as the fresh mass ( $\text{kg tree}^{-1}$ ) of the amount of pruned wood per tree each year individually weighed in the field. Canopy volume was annually calculated before pruning according to Hutchison (1978) based on canopy height and diameters (across and within rows) of the five central trees of each replicate. Likewise, the shaded area of the same trees was estimated in CTL, RDI and SDI as light intercepted at noon on completely clear days, when photosynthetic photon flux rate (PFR) was close to  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . PFR interception was calculated from 30 measurements corresponding to a grid ( $0.25 \text{ m}^2$  mesh) that covered half of the tree spacing, using a linear ceptometer with an 80 cm long probe (Accupar Linear PAR, Decagon Devices Inc., Pullman, WA, USA) on the soil surface. Cumulate shoot growth was measured in 4 marked current season shoots per tree, 2 trees per replicate each month with a tape measure (Tylon Pocket, Stanley, New Britain, CT, USA) in 2016 and 2017. At the beginning, at harvest and at the end of the growing season, trunk diameter of all trees was measured with a tape measure (Pi meter MF612A, Weiss, Erben-dorf, Germany). The measurements were always taken in a marked location in the trunk, at 0.20 m from the soil surface in the five central trees per replicate. Trunk cross-sectional area (TCSA) was estimated as the circle area from the trunk diameter measured.

## 2.7. Yield

At harvest (June 3<sup>rd</sup> and 10<sup>th</sup> in 2015, 17<sup>th</sup> and 22<sup>nd</sup> in 2016, 2<sup>nd</sup> in 2017 and 14<sup>th</sup> and 19<sup>th</sup> in 2018), fruits from 5 central trees of each

replicate were harvested and weighed. The individual yield of each tree was weighed to determine yield per tree. Similarly, at harvest, fruits were counted in 5 kg samples in order to calculate the unitary mass of the cherries. Double and cracked fruits in the sample were also counted in order to measure their proportion in the total yield. The number of fruit per tree was estimated from fruit unitary mass and yield per tree. Moreover, soluble solids concentration (SSC; %) was measured from 10 fruits per replicate with a refractometer (N1, Atago, Tokyo, Japan). With the aim of assess if irrigation treatments can affect fruit susceptibility to crack, cracking index was measured from the fruits harvested in 2018 following the procedure described by Christensen (1972). 50 fruits per replicate, four replicates per treatment, were immersed in 2 L distilled water (pH 7) at  $20^\circ\text{C}$ . Cracks presence on the fruit was evaluated after 2, 4 and 6 h. At each time, cracked cherries were removed and recorded. Cracking index was calculated as: Cracking index =  $100 \times [5a + 3b + c] \times (250)^{-1}$ . In this equation a, b and c represent the number of cracked fruits at 2, 4 and 6 h of immersion, respectively.

## 2.8. Statistical analysis

The experimental layout was a completely randomized block design with four replicates per treatment. Each replicate consisted of seven trees: the five central trees were used for measuring the yield and pruning wood per tree, while only the two central trees were used to monitor water relations, the other trees serving as guard trees. Analysis of variance (ANOVA) was performed using the statistical software packages IBM SPSS Statistic 24 (IBM Corp., Armonk, NY, USA) and Statgraphics centurion XVI (StatPoint Technologies Inc., The Plains, VA, USA) to determine the effect of the different irrigation treatments on soil and plant indicators, vegetative growth and yield. Means were separated by a post-hoc test (Duncan's multiple range) with a significance level of  $P < 0.05$ . Linear relationships as well as regression analysis between variables were calculated with SigmaPlot 12.5 (Systat software Inc., San Jose, CA, USA).

## 3. Results

### 3.1. Meteorological conditions and irrigation water applied

The reference crop evapotranspiration ( $ET_0$ ) showed a similar seasonal evolution all the years of the study (2015–2018), with an annual average sum of 1256 mm, with a maximum in June and July and mean values higher than  $5.0 \text{ mm d}^{-1}$  and daily peaks of  $7.0 \text{ mm d}^{-1}$  occurring in mid-late June. Precipitation was not sufficient to satisfy the demand of the crop's evapotranspiration varying from 260 to 360 mm. In 2015, precipitation was 129 mm during the growing season (April – September), which represented 49% of the annual total. This proportion changed in 2016 and 2017, when 142 and 84 mm of rain fell, representing 38 and 39%, respectively. During the pre-harvest period (April – June) of 2018, the rainfall recorded was higher than the rainfall measured during the three previous years for the whole growing season (264 mm). The daily maximum vapour pressure deficit (VPD) during the growing season showed a similar pattern to  $ET_0$ , with a VPD average of 1.6 kPa. The maximum annual values were always recorded in July, with the maximum VPD registered (4 kPa) on DOY 188 in 2015.

During 2015 pre-harvest, a hail storm (139 DOY) partially damaged the fruit and negatively affected the commercial yield. In 2018, 11 d before the first harvest, a rainy spell of 7 d (86 mm of rain) affected fruit quality. The occurrence of precipitation during the 2018 pre-harvest reduced the atmospheric demand in 2018 and consequently diminished the total water supplied (Table 1), although the pre-harvest period lasted longer than in the previous years.

Table 1 presents the irrigation water applied to each treatment and in each period (pre-harvest, floral differentiation and post-harvest) from 2015 to 2018. It can be seen that RDI saved the greatest amount of water (39%) compared with CTL over the whole experiment, while SDI

**Table 1**

Irrigation water applied ( $\text{m}^3 \text{ha}^{-1}$ ) during each period, pre-harvest (Pre), floral differentiation (Floral Diff) and post-harvest (Post) of the experimental period 2015–2018 to ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

		Irrigation water applied ( $\text{m}^3 \text{ha}^{-1}$ )			
		CTL	SDI	RDI	FRM
2015	Pre	1977	1629	1717	2522
	Floral Diff	570	561	567	421
	Post	4369	3114	1820	3336
	<b>TOTAL</b>	<b>6916</b>	<b>5304</b>	<b>4104</b>	<b>6279</b>
2016	Pre	2143	1527	1909	2689
	Floral Diff	666	635	640	539
	Post	4221	2668	1677	2889
	<b>TOTAL</b>	<b>7030</b>	<b>4830</b>	<b>4226</b>	<b>6117</b>
2017	Pre	1904	1379	1664	2189
	Floral Diff	673	662	661	539
	Post	4324	3091	2091	2954
	<b>TOTAL</b>	<b>6901</b>	<b>5132</b>	<b>4416</b>	<b>5682</b>
2018	Pre	1978	1405	1760	2201

and FRM saved 28% and 15% respectively, of the water applied to CTL.

**3.2. Soil water status**

The seasonal trends in soil water matric potential ( $\Psi_m$ ) and soil water content ( $\theta_v$ ) distinguished between the different irrigation strategies imposed in the three irrigation phases every year of the study.  $\Psi_m$  mean values at 25 and 50 cm depth were similar during pre-harvest in CTL, FRM and RDI treatments (between -10 and -30 kPa; Table 2) and all were higher than in SDI treatment (between -30 and -130 kPa; Table 2). During the first days of post-harvest (floral differentiation), there were no differences among treatments. Once the irrigation deficit was imposed in RDI and SDI treatments, differences appeared, and each treatment was significantly different.

As in the case of  $\Psi_m$ ,  $\theta_v$  showed significant differences among treatments during post-harvest all years of the study. The FRM treatment showed a significantly lower  $\theta_v$  value during 2017 floral differentiation, indicating that FRM changed its irrigation regime. The mean  $\theta_v$  value in CTL treatment remained in a close range (between 98 and 84% at 20 cm depth and 93 and 82% at 40 cm), while RDI was clearly influenced by irrigation changes (average of 88% and 56% during pre-harvest and post-harvest, respectively, Table 3).

**3.3. Plant water status**

**3.3.1. Midday stem water potential**

Midday tree water status was affected by irrigation treatment every year of the study. In the pre-harvest periods of 2015 and 2017, SDI trees resulted in significantly lower  $\Psi_{stem}$  than controls at all measurement times, except the first one; however, in 2016 and 2018 there were hardly any differences between treatments until just before harvest (Fig. 1).

As was to be expected, when the evaporative demand increased, all treatments, including CTL, exhibited a trend to lower values than during pre-harvest, however the steepest drop in  $\Psi_{stem}$  occurred in RDI and FRM in response to the deficit in soil water content following the irrigation treatments imposed. The reduction in  $\Psi_{stem}$  was clear between DOY 180 and DOY 240 (end June – early August), a change that occurred in parallel with the decrease in the soil water content (Fig. 1, Table 2). During 2017 post-harvest, the general seasonal trend of  $\Psi_{stem}$  was characterized by a steady, but more marked reduction, RDI trees resulted in  $\Psi_{stem}$  below -1.3 MPa at two consecutive measurement points. Similarly,  $\Psi_{stem}$  of FRM trees led to -1.8 MPa, which was the

**Table 2** Mean value of soil water potential ( $\Psi_m$ ) at 25 and 50 cm depth to each irrigation period, pre-harvest (Pre), floral differentiation (Floral Diff) and post-harvest (Post) of the experimental period 2015–2018 to ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

		$\Psi_m$ (kPa)				ANOVA	
		CTL	SDI	FRM	ANOVA		
2015	Pre	-11.27	-22.27	-10.36	-16.14	a	**
	Floral Diff	-11.65	-9.51	-12.10	-16.22	a	n.s.
	Post	-22.98	-93.90	-206.14	-29.61	a	*
2016	Pre	-11.07	-30.62	-10.99	-14.02	a	n.s.
	Floral Diff	-12.17	-14.21	-11.84	-21.41	a	n.s.
	Post	-22.53	-188.42	-273.77	-33.47	a	*
2017	Pre	-11.81	-61.22	-14.10	-22.76	a	**
	Floral Diff	-10.02	-13.56	-10.03	-14.11	a	n.s.
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2018	Pre	-12.93	-39.28	-14.58	-15.43	a	*
	Floral Diff	-11.81	-61.22	-14.10	-22.76	a	**
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2015	Pre	-11.27	-22.27	-10.36	-16.14	a	**
	Floral Diff	-11.65	-9.51	-12.10	-16.22	a	n.s.
	Post	-22.98	-93.90	-206.14	-29.61	a	*
2016	Pre	-11.07	-30.62	-10.99	-14.02	a	n.s.
	Floral Diff	-12.17	-14.21	-11.84	-21.41	a	n.s.
	Post	-22.53	-188.42	-273.77	-33.47	a	*
2017	Pre	-11.81	-61.22	-14.10	-22.76	a	**
	Floral Diff	-10.02	-13.56	-10.03	-14.11	a	n.s.
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2018	Pre	-12.93	-39.28	-14.58	-15.43	a	*
	Floral Diff	-11.81	-61.22	-14.10	-22.76	a	**
	Post	-34.39	-146.67	-335.85	-36.58	a	*
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	Post	-22.98	-93.90	-206.14	-29.61	a	*
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	Floral Diff	-12.17	-14.21	-11.84	-21.41	a	n.s.
	Post	-22.53	-188.42	-273.77	-33.47	a	*
2017	Pre	-11.81	-61.22	-14.10	-22.76	a	**
	Floral Diff	-10.02	-13.56	-10.03	-14.11	a	n.s.
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2018	Pre	-12.93	-39.28	-14.58	-15.43	a	*
	Floral Diff	-11.81	-61.22	-14.10	-22.76	a	**
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2015	Pre	-11.27	-22.27	-10.36	-16.14	a	**
	Floral Diff	-11.65	-9.51	-12.10	-16.22	a	n.s.
	Post	-22.98	-93.90	-206.14	-29.61	a	*
2016	Pre	-11.07	-30.62	-10.99	-14.02	a	n.s.
	Floral Diff	-12.17	-14.21	-11.84	-21.41	a	n.s.
	Post	-22.53	-188.42	-273.77	-33.47	a	*
2017	Pre	-11.81	-61.22	-14.10	-22.76	a	**
	Floral Diff	-10.02	-13.56	-10.03	-14.11	a	n.s.
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2018	Pre	-12.93	-39.28	-14.58	-15.43	a	*
	Floral Diff	-11.81	-61.22	-14.10	-22.76	a	**
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2015	Pre	-11.27	-22.27	-10.36	-16.14	a	**
	Floral Diff	-11.65	-9.51	-12.10	-16.22	a	n.s.
	Post	-22.98	-93.90	-206.14	-29.61	a	*
2016	Pre	-11.07	-30.62	-10.99	-14.02	a	n.s.
	Floral Diff	-12.17	-14.21	-11.84	-21.41	a	n.s.
	Post	-22.53	-188.42	-273.77	-33.47	a	*
2017	Pre	-11.81	-61.22	-14.10	-22.76	a	**
	Floral Diff	-10.02	-13.56	-10.03	-14.11	a	n.s.
	Post	-34.39	-146.67	-335.85	-36.58	a	*
2018	Pre	-12.93	-39.28	-14.58	-15.43	a	*
	Floral Diff	-11.81	-61.22	-14.10	-22.76	a	**
	Post	-34.39	-146.67	-335.85	-36.58	a	*

Each value is the mean of the 4 replicates. Mean values within the same period (row) followed by a different letter denote significant differences among treatments according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA column, \*, \*\*, \*\*\* refer to significant effect at  $P = 0.05$ , 0.01 or 0.001 respectively and n.s. to not significant.

**Table 3**  
Mean value of soil water content referenced to field capacity ( $\theta_v$ ) at 20 and 40 cm depth to each irrigation period, pre-harvest (Pre), floral differentiation (Floral Diff) and post-harvest (Post) of the experimental period 2015–2018 to 'Prime Giant' sweet cherry trees exposed to four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

		20 cm		40 cm		ANOVA	ANOVA	ANOVA			
		$\theta_v$	(%) FRM	$\theta_v$	(%) FRM						
Pre Floral Diff Post	2015	CTL	92.81	93.31	84.00	92.16	86.41	68.70	88.40	n.s.	
		SDI	89.15	92.00	89.15	94.90	88.00	82.73	90.57	n.s.	
		FRM	84.33	68.91	68.88	56.90	82.20	72.89	58.97	69.65	*
Pre Floral Diff Post	2016	CTL	95.28	94.01	86.64	91.25	87.45	72.41	88.25	n.s.	
		SDI	98.30	93.43	86.63	96.65	93.29	82.43	82.01	91.18	n.s.
		FRM	94.10	61.40	75.68	57.47	88.47	67.01	61.55	67.11	*
Pre Floral Diff Post	2017	CTL	90.72	90.98	71.96	88.94	84.44	59.83	84.28	a	
		SDI	87.08	72.42	87.08	90.40	82.80	70.04	88.02	a	
		FRM	89.45	59.53	77.24	53.33	85.95	67.60	53.21	57.70	b
Pre	2018	CTL	91.98	94.34	74.45	89.69	85.56	68.07	90.15	a	
		ANOVA		*							**

Each value is the mean of the 4 replicates. Mean values within the same period (row) followed by a different letter denote significant differences among treatments according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA column, \*, \*\* refer to significant effect at  $P = 0.05$  or  $0.01$ , respectively and n.s. to not significant.

absolute minimum value measured throughout the experiment. At harvest, the  $\Psi_{stem}$  of SDI trees was  $-0.7$  MPa every year of the study, which was between  $0.14$  and  $0.20$  MPa lower on average than in CTL trees for 2015–2018, with significant differences (Fig. 1).

### 3.3.2. Leaf gas exchange

During the 2015, 2016 and 2017 post-harvest periods, significant differences were detected for CTL and RDI in Pn and gs. Based on the results of stomatal conductance, gs was significantly higher in CTL trees during post-harvest than in RDI trees ( $196$  and  $110$   $\text{mmol m}^{-2} \text{s}^{-1}$ , respectively). Post-harvest net photosynthesis also differed significantly between CTL and RDI trees every year of the study. During post-harvest, Pn pointed to statistically significant differences between CTL and SDI in 2016 and 2017, while gs showed no such difference since Pn showed less variability than gs between measurements within the same treatment (Table 4).

During pre-harvest, despite the differences in the water supplied by irrigation to each treatment, trees from all treatments resulted in similar gs and Pn. Stomatal conductance at the floral differentiation stage of 2017 identified statistical differences between FRM and the rest of the treatments, which agreed with the significantly lower soil water content and lower  $\Psi_{stem}$  compared with the other treatments (Table 4).

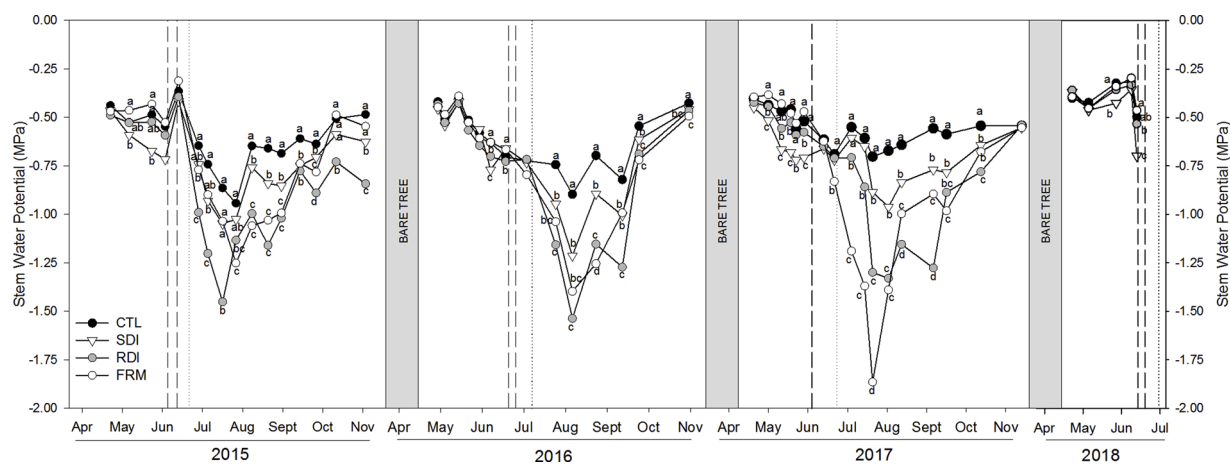
IWUE was higher in deficit treated trees than in controls. In 2015 pre-harvest all trees resulted in similar IWUE; however in 2016, 2017 and 2018 SDI trees turned out in higher IWUE than CTL trees but without significant differences. In post-harvest, RDI trees had an IWUE that was 30 and 23% higher than that measured in CTL and SDI trees, respectively, and, during post-harvest 2015, RDI trees were significantly more efficient in the use of water than all the other trees (Table 4).

### 3.3.3. Branch diameter fluctuations

The seasonal evolution of the MDS and BGR showed different results according to the irrigation treatment imposed. SDI trees recorded pre-harvest MDS values higher than  $400$   $\mu\text{m}$ , while well-watered plants of the other treatments showed values close to  $200$   $\mu\text{m}$ . During post-harvest, maximum MDS values were recorded in all the treatments in July. RDI produced the greatest fluctuations, higher than  $600$   $\mu\text{m}$ , and CTL trees reached fluctuations of  $320$   $\mu\text{m}$ . MDS of FRM trees was similar to the trees under RDI but not on the same dates (Fig. 2). The pre-harvest, floral differentiation and post-harvest means of  $SI_{MDS}$  in SDI trees were  $1.3$ ,  $1.05$  and  $1.3$ , in RDI trees were  $1.1$ ,  $1.05$  and  $1.6$ ; and in FRM trees were  $0.9$ ,  $1.2$  and  $1.5$ , respectively. In the 2017 post-harvest period, RDI trees showed a higher  $SI_{MDS}$  than in previous years, values reaching  $1.8$  at the end of July and early August. In the same vein, FRM led to a higher mean  $SI_{MDS}$  value of  $1.9$  in 2017, even exceeding  $3.0$  during mid-summer.

At the end of the experiment, the different irrigation treatments were seen to have induced clear differences in the branch growth rates of the trees. BGR showed sigmoid patterns each year of the study, with rapid vegetative growth that coincided with flower and fruit development in the tree (from March to June), slower growth from June to September, and no growth the rest of the season (October onwards). Mean seasonal branch growth in control trees was about  $4.3$  mm. Trees of deficit irrigation treatments resulted in a similar pattern but less pronounced, the BGR of the trees under SDI and RDI was 26 and 35% lower than that measured in CTL trees. During pre-harvest 2015 and 2017, BGR of trees was not significantly different among treatments; however, during 2016 and 2018 differences appeared, and SDI trees resulted in a significantly lower BGR than those from CTL (Fig. 2). The post-harvest irrigation deficit applied in RDI trees caused sharp decrease in its BGR. Deficit irrigation imposed during post-harvest in RDI trees resulted after 10 d in a BGR reduction from  $39$  to  $9$   $\mu\text{m d}^{-1}$ . Meanwhile, control trees remained BGR close to  $35$   $\mu\text{m d}^{-1}$ .





**Fig. 1.** Evolution of the midday stem water potential ( $\Psi_{\text{stem}}$ ) during the experimental period 2015–2018 of ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL, black circles), sustain deficit irrigation (SDI, triangles), regulated deficit irrigation (RDI, grey circles) and a treatment based on normal farming practices (FRM, white circles).

Vertical dashed lines show harvest days and dotted line the start of the postharvest deficit period. Each point is the mean value of 6 measurements. Different letters on the same day denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ).

### 3.4. Vegetative growth

Different irrigation strategies did not lead to significant differences in the tree’s vegetative growth for the first two years of the experimental period except shoot length. Tree’s TCSA was not significantly affected by irrigation treatments any year of study. On the other hand, tree’s pruned wood was significantly different among trees of different irrigation treatments (Table 5). In general, CTL trees increased their vegetative growth as the experiment progressed. RDI trees resulted in the lowest canopy volume in the last measurement of the experiment, and consequently, lower PFR interception and lower shaded area. Thus, in the last year of the study RDI trees had a significantly lower shaded area than CTL trees. Shoot length reached greater average values in the third year of the study in all the treatments, but especially CTL in which it coincided with a bigger pruning mass that year.

### 3.5. Yield and double and cracked fruits

There was no significant effect of irrigation on yield parameters (Fig. 3A and B). In 2015 and 2017, fruit yield and number of fruits per tree (Fig. 3B) were lower than in 2016 and 2018 in all treatments (with 23.5, 43.4, 29.6 and 40.3 kg tree<sup>-1</sup> for CTL trees each year of the study). It should be remembered that in 2015 as a result of a hail storm, 11% of that year’s commercial yield was damaged and could not be harvested. Consequently, fruit unitary mass was higher in 2015 and 2017 than in 2016 and 2018 and fruit size distribution resulted in greater proportion of fruits of SDI in the lowest categories, but with no differences among treatments (Figs. 3C and 4). Thus, high cropping years showed lower fruit unitary mass. A linear relationship was obtained between the total yield and the fruit unitary mass: [Unitary mass (g) = -0.1021 Yield (kg tree<sup>-1</sup>) + 13.67. ( $r^2 = 0.67$ )].

There were no significant differences in fruit SSC among treatments, although the deficit irrigation treatments tended to induce higher values, especially SDI, which, at the last harvest, led to average fruit SSC values that were 10% higher than that of controls (Fig. 3D). The frequency of double fruit was not influenced by the irrigation treatment and there were no significant differences due to this effect; the occurrence of double fruit varied from 1.5 to 13% of the total fruit harvested from year to year, depending on environmental conditions (Table 6).

Cracking incidence of cherries was not significantly different among treatments in 2017, a year in which, in the month prior to harvest, a rainfall episode of 11 mm caused percentage of cracking to reach 1% of the total fruit. In 2018, eleven days before harvest, several rain episodes took place with a total amount of 86 mm over seven days, and, depending on the treatment from 9 to 23% of the fruit cracked (Table 6). Cherries of CTL and FRM had a similar cracking incidence of close to 20% although SDI at the first harvest and both deficit treatments at the second harvest resulted in significantly less cracked fruit. These results agree with the cracking index determined in the laboratory, which was significantly higher in CTL and FRM cherries.

### 3.6. Water productivity

The productive efficiencies varied according to the irrigation imposed in each treatment. RDI trees resulted in higher water productivity (WP, calculated as the ratio of yield to irrigation water applied) than the trees of the other irrigation treatments, reaching 5.3 kg m<sup>-3</sup> in 2017, doubling the productivity of CTL trees (Table 7). Moreover, RDI trees tended to produce a greater number of fruits per trunk cross sectional area (fruit number efficiency, FE), and a greater number of fruits per increment of the trunk cross sectional area (fruit number per trunk increment, FTI). Furthermore, in 2018 RDI trees lead to significant higher yield efficiency (YE, ratio of yield to trunk cross sectional area) than controls (Table 7). Trees under RDI resulted in higher water productivity than the trees of any treatment every year of the study in spite of variations in yield. Trees under SDI also resulted in significant higher WP than those of CTL. However, these differences were not accompanied by differences in fruit and yield efficiency.

## 4. Discussion

The results of this study show that sweet cherry ‘Prime Giant’ seems to be sensitive to deficit irrigation during the pre-harvest period more than that post-harvest. The irrigation restrictions applied in RDI led to a statistically significant lower mean soil matric potential and soil water content than in CTL after harvest in this study (Tables 2 and 3). Moreover, SDI treatment, which enforced trees to a slight water deficit pre and post-harvest, resulted in statistically lower soil matric potential

**Table 4**  
Mean value of stomatal conductance (gs), net photosynthesis (Pn) and intrinsic water use efficiency (IWUE) to each irrigation period pre-harvest (Pre), floral differentiation (Floral Diff) and post-harvest (Post) of the experimental period 2015–2018 to ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

		gs (mmol m <sup>-2</sup> s <sup>-1</sup> )				Pn (μmol m <sup>-2</sup> s <sup>-1</sup> )				IWUE (μmol mol <sup>-1</sup> )						
		CTL	SDI	RDI	FRM	ANOVA	CTL	SDI	RDI	FRM	ANOVA	CTL	SDI	RDI	FRM	ANOVA
		Pre	175.6	188.9	192.8	179.5	n.s.	10.0	10.9	10.0	10.3	n.s.	57.1	57.7	51.7	57.2
Floral Diff	337.4	331.0	261.3	309.3	n.s.	17.3	18.7	15.7	17.1	n.s.	51.2	56.6	56.9	55.3	n.s.	
Post	211.0	175.7	119.7	154.6	bc *	16.1	14.1	11.8	11.7	b *	76.2	80.5	75.5	75.5	b *	
Pre	182.0	167.3	175.8	191.4	n.s.	14.5	14.8	15.3	15.0	n.s.	79.4	88.7	86.8	78.2	n.s.	
Floral Diff	274.0	229.0	342.5	283.8	n.s.	17.5	17.3	20.8	18.1	n.s.	63.8	75.5	60.7	63.8	n.s.	
Post	192.2	139.3	101.1	79.4	b *	15.6	11.5	10.9	8.8	bc *	81.2	82.7	108.0	110.5	a *	
Pre	178.8	173.1	184.3	182.5	n.s.	11.6	12.9	14.5	12.6	n.s.	68.4	74.3	68.5	69.1	n.s.	
Floral Diff	264.8	248.2	245.6	109.2	b *	19.4	17.6	17.5	13.0	n.s.	73.3	70.8	71.2	118.7	a **	
Post	185.7	149.0	109.7	84.5	c *	16.0	14.2	12.1	9.3	c *	86.3	95.6	109.9	109.9	a **	
Pre	191.1	180.7	184.5	194.7	n.s.	14.6	15.1	12.7	15.0	n.s.	76.4	83.8	69.1	77.0	n.s.	

Each value is the mean of the 4 replicates. Mean values within the same period (row) followed by a different letter denote significant differences among treatments according to Duncan multiple range test (P < 0.05). In the ANOVA column, \*, \*\* refer to significant effect at P = 0.05 or 0.01, respectively and n.s. to not significant.

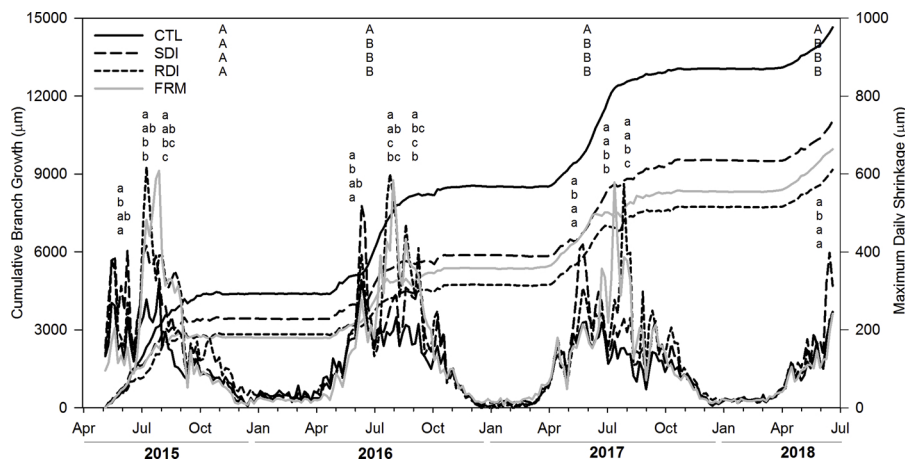
and soil water content than all the other irrigation treatments during the pre-harvest period in three of the four years for the Ψm (Table 2), but only in the last two years for θv (Table 3).

Consequently, these differences in water availability affected the plant water status. Ψ<sub>stem</sub> was a sensitive indicator for identifying tree water status according to the intensity of the water deficit applied. Ψ<sub>stem</sub> identified significant differences not only after harvest between RDI and CTL, when RDI provides only 55% of the water requirements, but also between SDI and CTL during pre-harvest, when a mild deficit is applied in SDI (Fig. 1).

gs has been reported to be highly dependent on Ψ<sub>stem</sub> and meteorological parameters in sweet cherry trees (Blanco et al., 2018). Thus, maximum gs and Pn annual values for all treatments coincided with pre-harvest and floral differentiation when evaporative demand rise and vegetative and reproductive sinks compete for carbohydrates. The pre-harvest period of ‘Prime Giant’ sweet cherry is short, lasts approximately 60 d (López-Ortega et al., 2017). Thus, during the last days of pre-harvest and floral differentiation, fruit growth, flower-bud differentiation, shoot extension and leaf growth coincide so there is competition for assimilates among the different processes (Yoon and Richter, 1990), making trees highly susceptible to water deficit during this time. An excessive water deficit in pre-harvest and floral differentiation would lead to stomatal closure, Pn decreases and consequently lower fruit and vegetative growth. The slight deficit irrigation applied in SDI trees during pre-harvest did not significantly decrease yield, but induced lower vegetative growth, especially in parameters such as current season shoot growth (Table 5). During post-harvest, when deficit irrigation was applied, RDI trees resulted in significantly lower gs and Pn than CTL trees every year of the study (Table 4), which suggests that in order to avoid excessive water losses sweet cherry trees regulate stomata closure in response to water stress. Pn showed significant differences between RDI and CTL only when deficit irrigation was applied in RDI. Pronounced and severe reductions of gs affected Pn, so slight water deficit did not cause Pn reductions. These results are consistent with those of Antunez-Barria (2006) and Marsal et al. (2009), who describe a drop in Ψ<sub>stem</sub> caused by deficit irrigation and reduced Pn in ‘Bing’ and ‘New Star’ trees. According to the results obtained, the values of gs (close to 100 mmol m<sup>-2</sup> s<sup>-1</sup>) and Pn (close to 10 μmol m<sup>-2</sup> s<sup>-1</sup>), during post-harvest had no negative effects on the following year’s yield and fruit quality (Fig. 3), although vegetative growth was affected (Table 5).

MDS increased as did the evaporative demand. During pre-harvest, MDS of CTL, RDI and FRM trees increased more than four times, among them FRM trees had the lowest MDS due to the high amounts of water available, especially in 2015 and 2016. MDS of SDI trees had a significantly higher increased of MDS during pre-harvest compared with the trees of the other irrigation treatments. When deficit irrigation was applied in the post-harvest period, the MDS of RDI trees rapidly increased, as can be seen in the SI<sub>MDS</sub> mean values recorded. SI<sub>MDS</sub> has been successfully used in irrigation management in fruit trees (Puerto et al., 2013). Absolute values of tree water status indicators are highly dependent of environmental conditions, canopy architecture, soil variability, etc. Consequently, replication of irrigation strategies is limited. In order to ease the replication of the irrigation strategies followed, the SI<sub>MDS</sub> mean values obtained according to tree phenology are provided. Thus, the trees whose SI<sub>MDS</sub> during pre-harvest was above 1.3 had lower current season shoot growth and tended to smaller fruit (Fig. 4, Table 5). On the other hand, during post-harvest, the trees with SI<sub>MDS</sub> values of around 1.6 had a lower canopy volume and produced less pruning wood, but this did not penalize the following year’s yield. It was also observed that FRM trees that were over-irrigated during pre-harvest (SI<sub>MDS</sub> = 0.9) did not give a higher fruit yield but increased the pruning wood, which increased crop management costs.

The effects of water deficit on tree’s vegetative growth could also be identified in the BGR. Similarly to MDS, BGR presented different values according to the irrigation regime (Fig. 2). However, as evaporative



**Fig. 2.** Evolution of the branch maximum daily shrinkage (MDS) and cumulative branch growth during the experimental period 2015–2018 of ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL, solid black line), sustain deficit irrigation (SDI, long dashed line), regulated deficit irrigation (RDI, short dashed line) and a treatment based on normal farming practices (FRM, grey line).

Represented values are the mean of 6 measurements during a period of 5 days. Different lower case letters denote significant differences among treatments to MDS, 10 days before harvest and 10 and 20 days after floral differentiation, and different upper case letters denote significant differences among treatments to cumulative branch growth at harvest, according to Duncan multiple range test ( $P < 0.05$ ). Both upper and lower case letters are (in order) from top to bottom, CTL the top letter, SDI the second top letter, RDI the third top letter and FRM the bottom letter.

demand increased in late pre-harvest, differences between the trees of the different irrigation treatment arose. These differences were more evident in 2016, a high cropping year. Thus, in the days immediately before the 2016 harvest, SDI trees had BGR 27% lower than CTL. Consequently, the highest BGR values were recorded each season in CTL from June to July ( $65\text{--}75 \mu\text{m d}^{-1}$ ), which is in line with the higher increase of TCSA measured in those trees (Table 5). Once the deficit irrigation was applied during post-harvest, BGR of RDI trees decreased sharply, which, at the end of the last season as a consequence of deficit irrigation, trees of RDI resulted in an accumulated BGR that was 1700 and 5500  $\mu\text{m}$  lower than the trees of SDI and CTL, respectively (Fig. 2). That is an important effect of deficit post-harvest irrigation on tree’s vegetative growth. Other authors have also reported that an irrigation deficit inhibits vegetative growth (Chalmers et al., 1981; Mitchell and Chalmers, 1982).

This cumulative effect of deficit irrigation on BGR was also noted in other vegetative growth indicators. Dehghanianji et al. (2007) reported the strong effect of deficit irrigation on sweet cherry canopy volume. In our experiment, in the second year RDI trees had already reduced tree canopy volume and PFR intercepted by 7% and 17%, respectively, compared with CTL trees; however, the differences were not statistically significant until the third year of the experiment. There were no significant differences in TCSA among treatments any year of the study. However, at the end of the experiment, irrigation effect on the TCSA of the trees was greater, TCSA of CTL trees during the experiment had grown by 79  $\text{cm}^2$  (23, 38 and 24% more than SDI, RDI and FRM trees, respectively, Table 5). TCSA increases were proportional to the water applied to each irrigation treatment. Neilsen et al. (2014) described irrigation management (amount and frequency) as one of the strongest factors in TCSA growth in sweet cherry trees. Annual shoot growth was sensitive to water restrictions when other vegetative growth indicators were not. SDI and RDI trees in 2017 produced half of the annual shoot growth of CTL trees. In sweet cherry, current season shoots grow throughout pre-harvest; however, sometimes it can last longer (first post-harvest days) although 80% of the shoot growth takes place while the fruit is growing (Ayala and Lang, 2015; Rivera et al., 2016); thus, SDI trees, which were the only trees that did not completely satisfy the water requirements during pre-harvest, produced the lowest annual shoot growth in 2017 (Table 5). These results match those reported by Livellara et al. (2011) and Podesta et al. (2010) in ‘Brooks and’ ‘Bing’ sweet cherry trees, who described current season shoot long as an early indicator of water reductions in pre-harvest. All

these differences in vegetative growth were consistent with the amount of wood pruned, which gradually increased in CTL trees, while significantly lower results were obtained for SDI, RDI and FRM trees for the last 2 years. Comparing the deficit treatments, SDI trees resulted in the lowest shoot growth, while RDI trees led to the smallest canopy volume, pruning wood, TCSA and lower shoot growth than control trees. Thus, RDI treatment had a greater impact on tree vegetative growth than SDI treatment, which agreed with the results mentioned above for soil and plant water deficit indicators.

The way in which deficit irrigation was applied in RDI and SDI, avoiding  $\Psi_{\text{stem}}$  values below  $-1.6 \text{ MPa}$  during post-harvest deficit irrigation, might be reason why fruit yields were not significantly penalized in the subsequent seasons. Marsal et al. (2009) proposed  $-1.5 \text{ MPa}$  as the  $\Psi_{\text{stem}}$  threshold value in post-harvest deficit irrigation so as not to affect the following season’s yield. However, in 2017 post-harvest, FRM trees produced a one-off  $\Psi_{\text{stem}}$  value below  $-1.8 \text{ MPa}$  that did not cause a significantly lower yield, although FRM was the least productive treatment (Fig. 3A). Although there were no significant differences among irrigation treatments in total or partial yield, number of fruit per tree or unitary fruit mass at harvest, SDI trees, especially at the first harvest, led to higher number of fruits per tree but of lower unitary fruit mass than CTL fruit, particularly in 2017 and 2018. Fruits of SDI trees were almost 1 g smaller than those from CTL trees (Fig. 3C). Lower unitary mass in sweet cherry fruit is closely related to lower crop profitability; and, although there were no significant differences in fruit unitary mass among treatments, there was a clear trend for SDI trees to produce fruit of the smallest categories, equatorial diameter  $< 28 \text{ mm}$  (Fig. 4). These results concerning SDI fruit unitary mass can be related to the significantly lower  $\Psi_{\text{stem}}$  values during pre-harvest, which suggests a closer relationship between lower fruit unitary mass and a decrease in plant water status during pre-harvest rather than during post-harvest (Fig. 1). The slight water deficit applied during early pre-harvest did not induce higher cherry run-off; on the contrary it could have induced a slightly higher number of fruit per tree which added to the water deficit applied during late pre-harvest (final phase of fruit development), affected fruit size. Consequently, the combined effect of higher number of fruit per tree and water deficit during fruit cell enlargement in SDI trees tended to produce fruit of smaller size (Fig. 3). Similarly, SDI fruit led to higher SSC than CTL fruit but without significant differences. Higher SSC values are typically associated with RDI treatments. In tart cherry, Papenfuss and Black (2010) also reported higher SSC values as a result of deficit treatments, but only described

**Table 5**  
Influence of four irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM) on the vegetative growth of 'Prime Giant' sweet cherry trees over the seasons (2015–2018).

	Pruning wood (kg tree <sup>-1</sup> )		Canopy volume (m <sup>3</sup> )			Photosynthetic Photon Flux Rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		Trunk Cross Section Area (cm <sup>2</sup> )			Shoot growth (m)						
	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017					
CTL	4.54	b	7.69	a	9.32	a	780	805	875	214	241	260	293	0.30	a	0.44	a
SDI	3.45	b	5.42	bc	5.33	bc	752	637	692	236	260	278	302	0.21	b	0.22	b
RDI	3.56	b	5.05	c	4.29	c	726	683	544	216	230	242	263	0.20	b	0.25	b
FRM	6.59	a	6.47	b	5.89	b	n.s.	n.s.	*	229	255	267	290	0.27	ab	0.34	ab
ANOVA	*	**	**	**	***	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	*	*	*

Each value is the mean of the 4 replicates. Different letters on the same parameter (column) denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA row, \*, \*\*, \*\*\* refer to significant effect at  $P = 0.05$ , 0.01 or 0.001, respectively and n.s. to not significant.

significant differences in the sustained deficit irrigation treatment which satisfied 30% of the ETC.

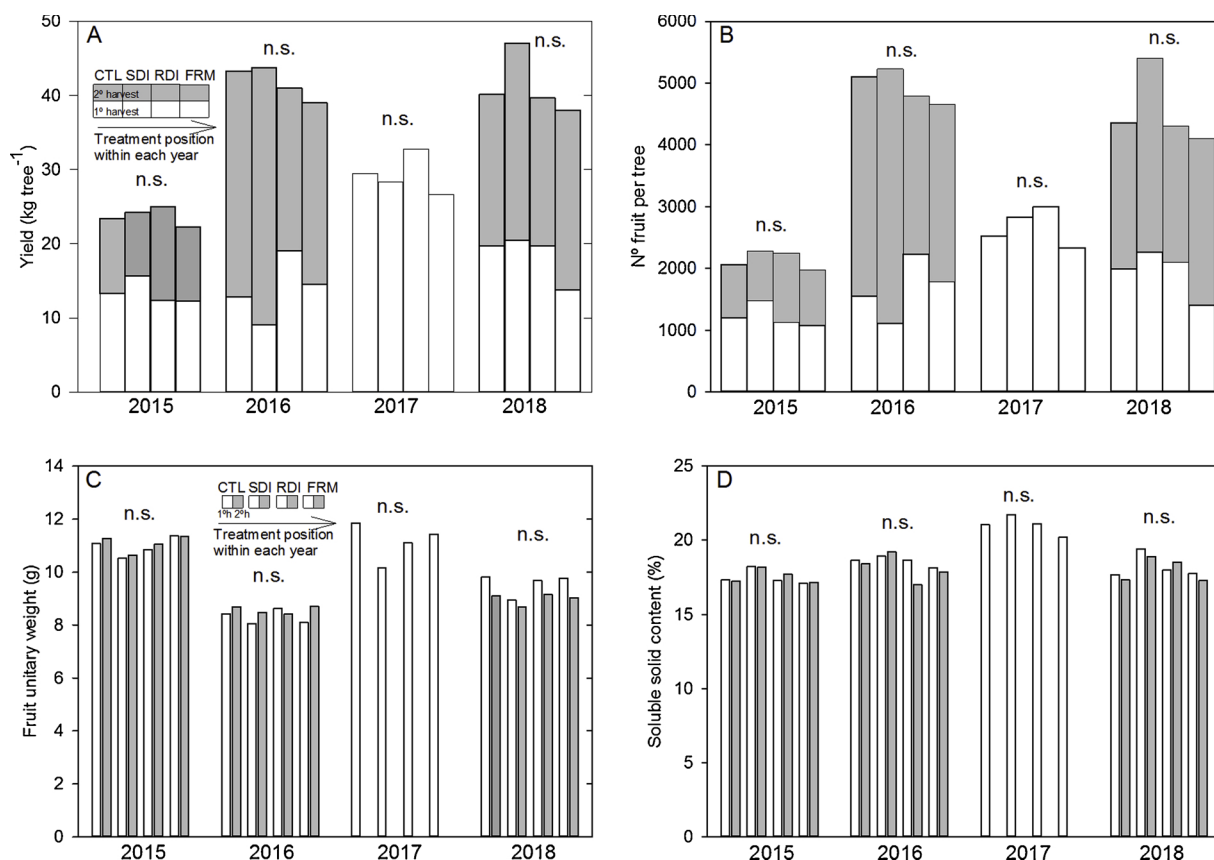
These effects of deficit irrigation on fruit mass could also be enhanced not only by water stress, but also by the different crop loads registered among seasons. Yield differences among years affected fruit unitary mass, and a high crop load itself was a factor affecting fruit mass at harvest (Fig. 3C). Thus, mean unitary fruit mass in 2016, the year with the largest crop load, was almost 3 g lower than in 2015 and 2017. However, in all harvests SDI trees lead to produce lower mean unitary fruit mass than CTL trees. The linear relationship between crop load and unitary mass obtained using the data for all years of the study suggests that the yield should not exceed 24–25 t ha<sup>-1</sup> if a unitary fruit mass of 10 g is to be achieved. Since sweet cherry price is positively correlated with fruit size, yields higher than 25 t ha<sup>-1</sup> will lower the price and consequently the profits of growers.

There was no clear influence of irrigation management in our growing conditions on the occurrence of double fruits. In crops such as peach or nectarine, post-harvest water deficit during summer has been demonstrated to increase the proportion of double fruits (Johnson et al., 1992; Naor et al., 2005). However, no such effect was evident in our experiment. Even in the 2017 post-harvest period, when soil water deficit indicators recorded minimum values (Table 2 and 3) and  $\Psi_{\text{stem}}$  fell below -1.8 MPa in FRM (Fig. 1), neither RDI nor FRM led to significantly more double fruits than CTL. However, in 2018 SDI treatment resulted in a higher proportion of double fruits (9%) than the other treatments. Beppu et al. (2001) and Roversi et al. (2008) reported that high temperatures during flower differentiation, especially during sepal to petal differentiation, might cause greater incidence than water deficit.

In both 2017 and 2018, cracked fruit were recorded at harvest. It is well known that rain-induced cracking is the major cause of crop loss in sweet cherry (Correia et al., 2018) and that sensitivity to fruit cracking is highly dependent on the cultivar. According to our results, 'Prime Giant' can be considered sensitive to fruit cracking (Table 6). In 2018, several rain episodes prior to harvest caused a loss of total yield with differences observed among treatments. Fruit of SDI and RDI resulted in a lower incidence of rain-induced cracking. This behaviour might be related with fruit lower water content and thicker skin in fruit of deficit irrigation treatments, as thicker fruit cuticle has been related as an effect of deficit irrigation on fruit (Pérez-Pastor et al., 2007). These results were consistent with the cracking index calculated in the laboratory, where RDI and SDI fruit were seen to be less likely to crack (Table 6). The result of a lower cracking index is a longer shelf-life, since fruit prone to cracking are also prone to developing diseases during storage (Zoffoli et al., 2017).

Of the four irrigation strategies assayed, RDI led to the greatest WP every year of the study (Table 7). SDI treatment also exhibited significant differences with CTL. A higher WP in deficit irrigation treatments has been reported in other crops such as peach, citrus and almond (Ghrab et al., 2013; Gonzalez-Altozano and Castel, 1999; Puerto et al., 2013).

RDI trees tended to higher FE than all the other trees, however, there were no significant differences among irrigation treatments any year of the study. Regarding YE, there were significant differences among treatments in 2018. RDI trees were the most productive trees per trunk section, whereas FRM and SDI trees did not result in statistical higher YE than CTL trees (Table 7). Significant differences in FTI and YE were due to the effect of the irrigation treatment on trunk growth more than on yield. Even though there were no differences in yield among treatments, the TCSA in CTL increased during the experiment by 10% more than in RDI (Table 5). The YE results obtained were similar to those reported by Nieto et al. (2017) but lower than those of Larsen et al. (1987) for adult trees. These lower results could be due to the rootstock used (SL64). SL64 has been reported by López-Ortega et al. (2016) and Aglar and Yildiz (2014) as a rootstock that produces low YE in sweet cherry cultivars such as 'New Star' and '0900 Ziraat'.



**Fig. 3.** Influence of four irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM) on ‘Prime Giant’ sweet cherry yield (A), number of fruits per tree (B), fruit unitary weight (C), and soluble solid content (D) over the harvest (2015–2018).

Each value is the mean of the 4 replicates. n.s. denotes no significant differences among treatments neither total nor partial harvests within each year according to ANOVA ( $P < 0.05$ ). Within each year treatments are, in order, from left to right CTL, SDI, RDI and FRM. First and second harvests are white and grey colored, respectively.

### 5. Conclusion

A water saving of 39% with RDI did not penalize total fruit yield or quality, particularly fruit size. The regulated water deficit imposed during post-harvest in RDI trees decreased stomatal conductance and stem water potential, which resulted in lower vegetative growth than obtained in CTL trees. Similarly, SDI treatment, which saved 28% of the water applied compared with CTL treatment, provided similar yields and lower vegetative growth. However, SDI trees tended to produce

smaller fruits, which would negatively affect grower’s profits. Therefore, as long as there is water available during the pre-harvest period, even slight water deficits must be avoided. Fruit of both water deficit treatments led to similar SSC and lower cracking susceptibility than CTL and FRM fruit, which could be a key factor for storage and shelf-life. It was seen that the vegetative growth of sweet cherry trees exposed to post-harvest water deficit was more affected than reproductive growth.

**Table 6**

Percentage of double and cracked fruits and cracking index of ‘Prime Giant’ sweet cherry fruit from four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

	Double fruit (%)				Cracked fruit (%)				Cracking Index				
	2015	2016	2017	2018	2017	2018				2018			
						1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest	1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest	1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest		
CTL	11.6	2.3	2.4	5.5	1.5	22.9	a	23.9	a	57	a	66	a
SDI	14.9	1.6	1.5	9.2	0.6	9.3	b	9.7	bc	42	b	47	b
RDI	12.2	1.7	0.9	5.0	0.7	14.8	ab	8.1	c	47	b	49	b
FRM	14.6	2.0	1.3	6.2	1.3	20.9	a	18.0	ab	64	a	67	a
ANOVA	n.s.	n.s.	n.s.	n.s.	n.s.	*		**		*		*	

Each value is the mean of the 4 replicates. In cracked fruit and cracking index, first and second harvest of 2018 were differentiated. Different letters on the same parameter and year (column) denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA row, \*, \*\* refer to significant effect at  $P = 0.05$  or  $0.01$ , respectively and n.s. to not significant.

Table 7

Irrigation water productivity (WP), fruit efficiency (FE), fruits per trunk increment (FTI) and yield efficiency (YE) over the seasons (2015–2018) to ‘Prime Giant’ sweet cherry trees exposed to four different irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM).

	WP (kg m <sup>-3</sup> )			FE (fruits cm <sup>-2</sup> trunk)			FTI (fruits cm <sup>-2</sup> trunk increment)			YE (kg cm <sup>-2</sup> )								
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018						
CTL	2.52	c	1.79	c	2.37	c	21.15	9.73	14.41	200.3	b	279.8	130.0	b	0.179	0.113	0.135	b
SDI	3.51	b	2.58	ab	3.80	b	20.51	10.17	17.94	226.1	b	334.0	315.1	a	0.170	0.103	0.157	ab
RDI	4.47	a	3.87	a	5.33	a	21.17	12.66	22.20	329.2	a	338.6	248.2	a	0.178	0.138	0.203	a
FRM	2.66	bc	2.09	bc	3.92	b	18.35	8.74	18.78	183.6	b	188.8	240.3	a	0.154	0.094	0.177	ab
ANOVA	**	***	***		n.s.	n.s.	n.s.		*	*	n.s.	**		n.s.	n.s.	n.s.	*	

Each value is the mean of the 4 replicates. Different letters on the same parameter and year (column) denote significant differences among treatments, according to Duncan multiple range test ( $P < 0.05$ ). In the ANOVA row, \*, \*\*, \*\*\* refer to significant effect at  $P = 0.05$ , 0.01 or 0.001, respectively and n.s. to not significant.

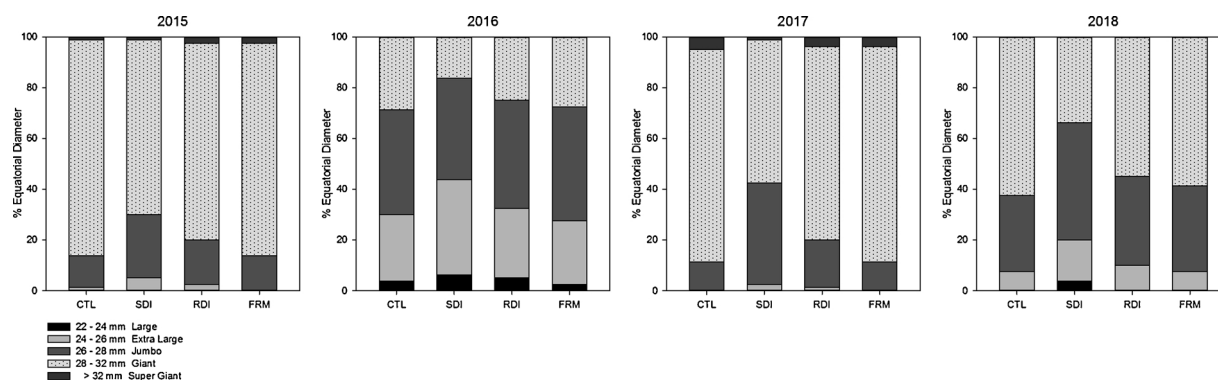


Fig. 4. Fruit size distribution at harvest (2015–2018) of ‘Prime Giant’ sweet cherry trees subjected to four irrigation treatments, control (CTL), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and a treatment based on normal farming practices (FRM). Each distribution is the mean of 20 fruit per replicate, and 4 replicates per treatment.

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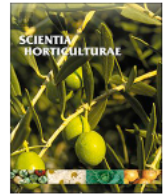
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Article IV

High tunnel cultivation of sweet cherry (*Prunus avium L.*): physiological and production variables.

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# High tunnel cultivation of sweet cherry (*Prunus avium* L.): physiological and production variables

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## ABSTRACT

Use of high tunnels in sweet cherry production is a popular way to reduce rain-induced fruit cracking. However, little information is available for its effects on tree physiology and fruit quality in established sweet cherry orchards. The aim of this study was to evaluate changes in the physiological and production variables of sweet cherry under high tunnels in a Mediterranean type climate in the Central Valley of Chile (35° 1' S, 71° 32' W). In 2017, a trial was conducted in a commercial orchard with the early and highly-productive cultivar combination of 'Royal Dawn' on 'MaxMa 14'. Trees were trained as a Y-trellis, spaced at 4.5 x 2.0 m and drip irrigated with four emitters (each emitter of 4 L h<sup>-1</sup>) per tree. Two treatments of five blocks per treatment were imposed. Treatments were 'covered' = trees under multi-bay Haygrove® high tunnels and 'open' = trees under open field conditions (control). The average value of g<sub>s</sub> increased during fruit development but the values under cover were generally 30% higher than in the open. Full bloom occurred 4 days earlier under cover and fruit was harvested 8 days earlier than that in the open and yields were similar (15 kg tree<sup>-1</sup>). Trees under high tunnels received 20% less amount of water than uncovered trees. There were no differences in fruit quality from different canopy layers. Significantly less cracking losses (3%) were observed in covered trees compared with open (19%). No differences in the ratio of leaf area to fruit number (193 cm<sup>2</sup> fruit<sup>-1</sup>) were found between covered and open trees but fruit from covered trees were larger, less sweet and softer in firmness than that from trees in the open. These altered fruit quality attributes should be improved in relation to the target market and the associated requirements for transport and storage. Results indicate that the use of high tunnels for 'Royal Dawn' on 'MaxMa 14' sweet cherry production, in a Mediterranean type climate, reduces susceptibility to rain-induced cracking, advances the time of harvest and increases fruit size.

## 1. Introduction

In most parts of the world where sweet cherries are grown, rain-induced fruit cracking (rain cracking) is the main limitation to production, as it can seriously reduce commercial return (Correia et al., 2018). The early, large and firm sweet cherry cultivars, such as 'Brooks' and 'Royal Dawn', are particularly susceptible to rain cracking and often suffer major (> 40%) or catastrophic losses (Simon, 2006). During rain cracking, water uptake by the fruit leads to the mechanical failure of the strained skin (Brüggenwirth and Knoche, 2016). The basic mechanisms of rain cracking are still uncertain. Factors inducing rain cracking have been extensively reviewed (Knoche and Lang, 2017; Knoche and Winkler, 2017; Meland et al., 2014; Sekse, 1995; Simon, 2006) and the use of physical barriers covering the fruit (Jung et al., 2016; Sotiropoulos et al., 2014) and/or protection of the whole tree

have been proposed as the primary techniques to overcome this problem in commercial orchards (Børve et al., 2003; Blanke and Balmer, 2008; Lang, 2009).

Among the different options for rain covers, high tunnels have been proposed as a best option for reducing fruit cracking and the added capital expense of the tunnel structure is justified (Lang, 2009). High tunnels are structures about 2-3 m in height, constructed of metal hoops with single-bay and multi-bay designs (Lamont, 2009). Multi-bay complexes are more suitable for tree fruit, as these are large enough to allow normal canopy management (Janke et al., 2017). Most high tunnels are neither heated nor cooled artificially (Lamont, 2009). High tunnels are covered by flexible and removable plastic films. Plastic covers maintain their properties for a certain amount of time, so they should be periodically replaced under the supervision of a waste manager to avoid environmental impact (Kyrikou and Briassoulis,

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2007).

It has been reported that high tunnels influence microclimate conditions, decreasing light levels and increasing air temperature and relative humidity (Ortiz et al., 2012). Plastic covers transmit most solar radiation but prevent or limit convective and radiative thermal transfer to the outside, so energy is retained and heat accumulates (Jett, 2017; Castilla, 2007). As a consequence of air temperature increase there is faster accumulation of growing degree days and hours, base temperature 4 °C (Retamal-Salgado et al., 2015). Severe overheating is prevented by ventilation. This is achieved either by taking out the side-walls or by rolling up the plastic sheets when it is not raining – which is a labor-intensive and expensive activity (Knewton et al., 2012; Lamont, 2009; Lamont et al., 2002; Meland et al., 2014).

The responses of annual plants (e.g. tomato *Solanum lycopersicum* L., pepper *Capsicum annuum* L., strawberry *Fragaria x ananassa*, raspberry *Rubus idaeus*) to plastic covering have been widely studied (Carey et al., 2009; Hanson et al., 2011; Lamont, 2005; Singh et al., 2012). However, similar information is scarce for deciduous fruit trees. Compared to open-field cultivation, the use of high tunnels advances harvest by 7 to 21 days in tomato, pepper, eggplant *Solanum melongena* L., zinnia *Zinnia elegans* L. and snapdragon *Antirrhinum majus* L. (Zhao et al., 2014). High tunnels similarly advance harvest by between 7 and 14 days in persimmon *Diospyros kaki* L. (Mason et al., 1992), table grapes *Vitis vinifera* L. (Novello and de Palma, 2008) and sweet cherry (Blanke and Balmer, 2008). The changed microclimate in high tunnels also has direct effects on the production and physiological responses of peach *Prunus persica* L. (Layne et al., 2013), mandarin *Citrus unshiu* Marc (Nesbitt et al., 2008) and blueberry *Vaccinium corymbosum* L. (Ogden and van Iersel, 2009). It has been reported that inside a high tunnel, air temperatures are between 3 and 15 °C higher than in the open (Black and Drost, 2010).

Recent reports indicate that bloom and fruit development of sweet cherry may be ‘forced’ under protected cultivation, with the crop harvested earlier in the season and so perhaps able to attract a higher price (Hecher et al., 2014; Meland et al., 2017; Overbeck et al., 2018). Experience with a new planting of sweet cherry under high tunnel, found increases in leaf area and in terminal shoot growth and reduced disease incidence, so reducing pesticide use (Lang, 2009). Growing sweet cherry under high tunnels may also be an effective way of avoiding damage from spring frosts during bloom and fruit set. These benefits are in addition the exclusion of rain and thus a possible reduction in rain-cracking. In addition, high tunnels may mitigate adverse effects of extreme weather such as heavy rains, frosts and hails during fruit set and maturation (Lang et al., 2016). However, the reduced light interception (15 to 20% lower) and increased temperatures (3 to 15 °C higher) and increased relative humidities (RH, 6 to 20%) inside a high tunnel are likely to have negative effects on fruit growth and quality (Lang, 2014; Meland et al., 2017).

In consequence, we hypothesize that high tunnels will not only reduce sweet cherry rain cracking but will also have positive- and negative-going influences on harvest time and fruit quality. The aim of this research was to study the use of high tunnels in an already-established sweet cherry orchard under a temperate Mediterranean type climate.

## 2. Materials and methods

### 2.1. Plant material and environmental conditions

The study was carried out during the 2017–2018 season in an established commercial orchard using the sweet cherry cultivar combination of ‘Royal Dawn’ on ‘MaxMa 14’ trees with ‘Lapins’ as the pollinator. The orchard was established in 2010 in Palquibudi, Maule Region, Chile (35° 1’ S, 71° 32’ W). Trees were drip-irrigated and trained as a Y-trellis with scaffolds disposed at a 45° angle. Between-row tree spacing was 4.5 m and the in-row spacing was 2.0 m.

The climate is a temperate Mediterranean one, with hot, dry summers and mild, wet winters. The average annual rainfall does not

exceed 500 mm yr<sup>-1</sup>. This is largely distributed between May and July (winter) with erratic but potentially heavy rainfall in spring (Sep–Nov) that can exceed 30 mm d<sup>-1</sup>. Annual average temperature is 15 °C with daily average maximum and minimum temperatures of 30 °C (Dec) and 2 °C (July), respectively. The frost-free period extends from Nov to Mar. Daily meteorological records of rainfall and crop reference evapotranspiration, were provided by a weather station located 9 km from the orchard (FDF - Agroclima, Chilean consulting service to fruit growers). The soil is characterized by a clay loam texture and is well drained and permeable. The irrigation water source (average electrical conductivity, EC<sub>25°C</sub>, 1 dS m<sup>-1</sup>) was the Lontue River.

The trees of the both treatments were exposed to the same horticultural practices to assure uniform quality. Drip irrigation was provided by a double drip-line per row and four pressure-compensated emitters per tree (each emitter of 4 L h<sup>-1</sup>). Irrigation commenced in Sep before bloom and finished in late May (near leaf drop). Irrigation scheduling was according to tree water demand, based on observations of soil water content in soil pits. Irrigation frequency varied from one irrigation event per week early in the season (Sep) to three per week before harvest (Oct–Nov). Each irrigation event consisted of a single daily irrigation of 12 h. The aim of this irrigation management was to satisfy the water requirements of the crop and also ensure adequate availability of soil oxygen to allow normal physiological activity in the root.

Trees were pruned in Jan 2017 and spur thinning (35%) was carried out in winter (Jul 2017). A total of 10 bumble bee hives per hectare were placed inside and outside the tunnels at 10% bloom. In the high tunnels, full bloom occurred on Sep 11, four days earlier than outside.

### 2.2. Treatments

Two treatments were used in a uniform area of 3.5 ha. These were ‘covered’ trees under multi-bay high tunnels and ‘open’ trees under open field conditions (control). The treatments were distributed in a completely randomized block design with five blocks or replications (i.e., five high tunnels). Each block consisted of a high tunnel covering three adjacent rows of 50 trees each (150 trees). All measurements were made on the three central trees of the central row. The open treatment (control) was similarly arranged except that the trees were not covered.

The covered trees were established under multi-bay high tunnels (Pioneer model of Haygrove, Haygrove Ltd, Ledbury, United Kingdom). The high tunnel comprised an arched metal frame 9 m wide, 100 m long and 2.2 m side-wall height, with a maximum height of 4.9 m at the highest point (Lang, 2009). The multi-bay tunnels were covered with a polyethylene film (150 μm thick), 87% PAR transmission capacity, > 90% light diffusion and 85% thermicity (Luminal-Visqueen, bpi.films, London, United Kingdom). The polyethylene film was flexible enough to be rolled up manually only when midday temperatures exceeded 25 °C during bloom or later. The high tunnels were closed when temperatures fell below 10 °C in the evening. The polyethylene film was mounted over the tunnel structure 60 d before full bloom and 5 d after the application of hydrogen cyanamide (NH<sub>2</sub>CN, 2% Dormex BASF, Chile). High tunnels remained completely closed until three days after full bloom (DAFB) (day of year, DOY = 257), when sidewalls were opened for ventilation. On rainy days (DOY 273, 278, 302, 307, and 308) the high tunnels were closed manually. The film was rolled back after leaf drop (late May).

### 2.3. Field measurements

#### 2.3.1. Environmental monitoring

Temperature (°C) and RH (%) were recorded at 15 min intervals using remote - sensing (HOBO U12-012, Onset Computer Corporation, Bourne, MA, USA). The sensors were placed in three blocks at heights of 0.75 and 2.0 m on selected trees. The procedure was repeated for trees in the open. From the temperature and RH data, the vapor pressure

deficit (VPD, kPa) was calculated according to Allen et al. (1998). Similarly, soil volumetric water content ( $\theta_v$ ) was obtained using three-frequency domain reflectometry sensors (GS3, Decagon – Meter Inc., Pullman, WA, USA) located beneath the canopy, 1.0 m from the trunk and at depths of 25 and 60 cm. Values of  $\theta_v$  were referenced to field capacity ( $\theta_{vFC}$ , %).

### 2.3.2. Tree measurements

Tree water status was determined weekly in two trees per block and between 12:00 and 13:30 h (solar time) by measuring stem water potential ( $\Psi_{stem}$ , MPa) using a Scholander pressure chamber (Pump-Up Chamber, PMS Instrument Company, Albany, OR, USA). Values of  $\Psi_{stem}$  were obtained from shaded, healthy, mature leaves close to the trunk taken from the north quadrant. The selected leaves had previously been wrapped in small black polyethylene bags and covered with aluminum foil for at least 2 h prior (McCutchan and Shackel, 1992).

Stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) was measured using a portable porometer, model SC1 (Decagon – Meter Inc., Pullman, WA, USA) on four sun-exposed spur leaves from the outer canopy at midday using the same trees in which  $\Psi_{stem}$  was measured. Stomatal conductance and stem water potential were measured simultaneously in each block.

To measure fruit and vegetative growth, each tree canopy was divided into three layers, bottom (0.8 m), middle (1.5 m) and top (2.0 m). Three extension shoots and three fruit per canopy layer were randomly sampled from each block. Fruit diameter (diam, mm) was measured weekly from 13 DAFB until harvest (56 DAFB covered, 60 DAFB open) using digital calipers (Electronic Digital Caliper, Veto, Santiago, Chile). At the same times, extension shoot length and leaf number were also recorded. Fruit and leaf growth were characterized each week based on measurements of five representative fruit per canopy layer and 20 fruiting spurs, which were sampled between fruit set (6 DAFB covered or 8 DAFB open) and harvest (56 DAFB covered 60 DAFB open) from each replicate. Fruit were measured for size (diam, mm), and unitary weight (g) and soluble solid concentration (SSC, %). Weight was measured using a 0.001 g precision scale (GRAM, Labtech 1500, Ontario, Canada). Fruit absolute growth rate (AGR,  $\text{g d}^{-1}$ ) was estimated according to Retamal-Salgado et al. (2015). Values of SSC were measured using a digital thermo-compensated refractometer (PR32, Atago, Tokyo, Japan) from 29 DAFB to harvest.

Leaf areas (LA) were measured using a leaf area meter (LI-COR LI-3100, Lincoln, NE, USA). The total number of spurs and extension shoots and fruit numbers per tree were measured for one tree per block for each treatment. The LA of individual spurs and extension shoots were measured on the same trees to estimate whole canopy LA and the ratio leaf area/fruit number (LA/F) according to Whiting and Lang (2004).

### 2.3.3. Fruit quality at harvest

The commercial harvest date (covered DOY 310, 56 DAFB and open DOY 318, 60 DAFB) skin color was determined by the fruit skin color chart (cherry color chart scale, Pontificia Universidad Catolica de Chile). Five trees per treatment were harvested and the fruit from each canopy layer were evaluated separately. The yield (kg) of each canopy layer was recorded (Digital150, Grantech, Santiago, Chile). In addition, 100 fruit per canopy layer per tree (300 fruit in total per tree) were collected for quality determination. Fruit quality parameters included fruit size (mm), fruit weight (g), color (cherry color chart scale, Pontificia Universidad Catolica de Chile), SSC (%), PR32, Atago, Tokyo, Japan), titratable acidity (TA) ( $\text{g L}^{-1}$ , Titromatic - compact titrator; Crison, Barcelona, Spain) and fruit firmness. Fruit firmness was measured over the range 0 (soft) to 100 (firm) using a durometer (type A, Durofel Agrotechnologie, Tarascon, France) with a 2.5 mm tip. The maturity index (MI) was calculated as the ratio SSC/TA.

At harvest, fruit showing visible cracking while still on the tree was recorded for each canopy layer in each tree in each treatment. Three

types of cracking were considered - stem end, cheek and stylar end (Jung et al. 2016). Moreover, in order to assess fruit cracking potential, the cracking index (CI) was determined at laboratory 24 h after harvest as described by Christensen (1972) by immersion in tap water (unadjusted for pH; pH 7) and in an acidic medium (pH 4; 0.1 M citric acid and 0.2 M disodium phosphate solutions in a ratio 62:38) for 12 h (Zoffoli et al., 2008). This measurement used 50 fruit per canopy layer, from five trees per treatment (50 fruit per layer x 2 layers x 5 trees x 2 treatments = 1000 fruit). Fruit were examined for cracking after immersion for 2, 4 and 6 h. At each time, the numbers of cracked fruit was recorded and the cracked fruit were discarded. The cracking index was calculated as:  $CI = (5a + 3b + c) / (250)$ , where: a, b and c were the numbers of cracked fruit after 2, 4 and 6 h of immersion, respectively.

### 2.4. Statistical analyses

Analysis of variance, ANOVA, ( $p = 0.05$ ) was carried out to determine differences among treatments. ANOVA was followed by a Duncan's test. Treatment interactions were evaluated using a multivariate general linear model. Statistical analyses were carried out using IBM SPSS Statistics v24 (Armonk, New York, USA).

## 3. Results

### 3.1. Environmental monitoring

Preharvest temperatures recorded under the covers and in the open (control) followed similar general patterns but were significantly different (Fig. 1A). The maximum daytime temperatures under cover (at 2.0 m) were between 5 and 10 °C higher than in the open (Fig. 1A.1). The highest maximum temperature (36.3 °C) under cover occurred at Stage III of fruit development (297 DOY; 44 DAFB). However, during the period between bloom and fruit set, maximum temperatures under cover did not exceed 30 °C (Fig. 1A.1). On the other hand, the highest maximum temperature (33.5 °C) in the open was recorded at late Stage III (314 DOY; 57 DAFB).

Frost damage was not observed either under cover or in the open. The minimum nighttime temperatures under cover were between 1 and 3 °C higher than in the open (Fig. 1A.2). In the open, the lowest minimum temperature (1.4 °C) was recorded at Stage I of fruit development (279 DOY; 21 DAFB). Throughout fruit development, the lowest minimum (nighttime) temperatures under cover were always above 4 °C.

The RH measurements showed similar general patterns under cover and in the open. Values were slightly higher under cover (Fig. 1B).

VPD values under cover were generally 20% higher than in the open during Stage I of fruit development. Under the covers, non-limiting conditions of  $\theta_{vFC}$  at 25 cm and 60 cm depths were observed throughout fruit development (Fig. 2A and B). Here, maximum values of  $\theta_{vFC}$  were reached after irrigation and minimum seasonal values of 75% were recorded from 257 to 261 DOY. On the other hand, in the open  $\theta_{vFC}$  reached maximum values after a rain event (41.6 mm, 277 DOY) and minimum values below 60% (25 cm depth) on DOY 312 and 70% (60 cm depth) DOY 314.

### 3.2. Tree measurements

During Stage I and Stage II of fruit development (i.e., mid-September to early November), the pattern of  $\Psi_{stem}$  was similar for both treatments (Fig. 3A). However, significant differences in  $\Psi_{stem}$  between treatments were observed close to harvest (Fig. 3A). Trees under cover were harvested on DOY 310 (56 DAFB) when evaporative demand was low (VPD = 0.69 kPa) and  $\Psi_{stem}$  (-0.50 MPa) was significantly higher than in the open ( $\Psi_{stem}$  = -0.58 MPa). In contrast, trees in the open were harvested on DOY 318 (60 DAFB) when evaporative demand was high (VPD = 1.37 kPa). At 60 DAFB trees in the open showed limited

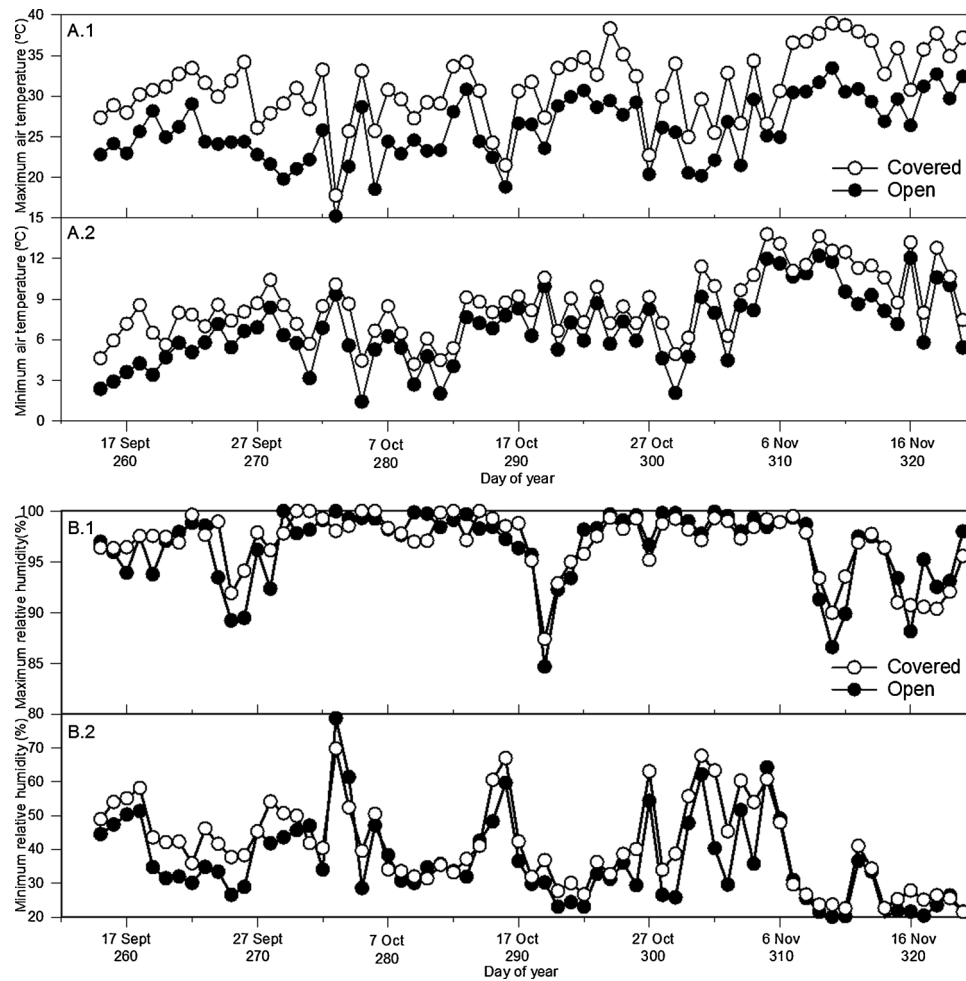


Fig. 1. (A.1) Daily maximum and (A.2) minimum air temperatures (°C) and (B.1) maximum and (B.2) minimum relative humidity (%) recorded at 2 m in ‘Royal Dawn’ on ‘MaxMa 14’ sweet cherry under cover and in the open.). Each data point represents the mean of three blocks (replications).

soil water availability ( $\theta_{vFC} < 75\%$ ;  $\Psi_{stem} = -0.92$  MPa), indicating water stress under open conditions.

treatments with significant differences since early Stage I of fruit development, DOY 278 (20 DAFB in the covered trees and 16 DAFB in the open trees) (Fig. 3B). The average value of  $g_s$  increased during fruit

Stomatal conductance ( $g_s$ ) showed an increasing pattern in both

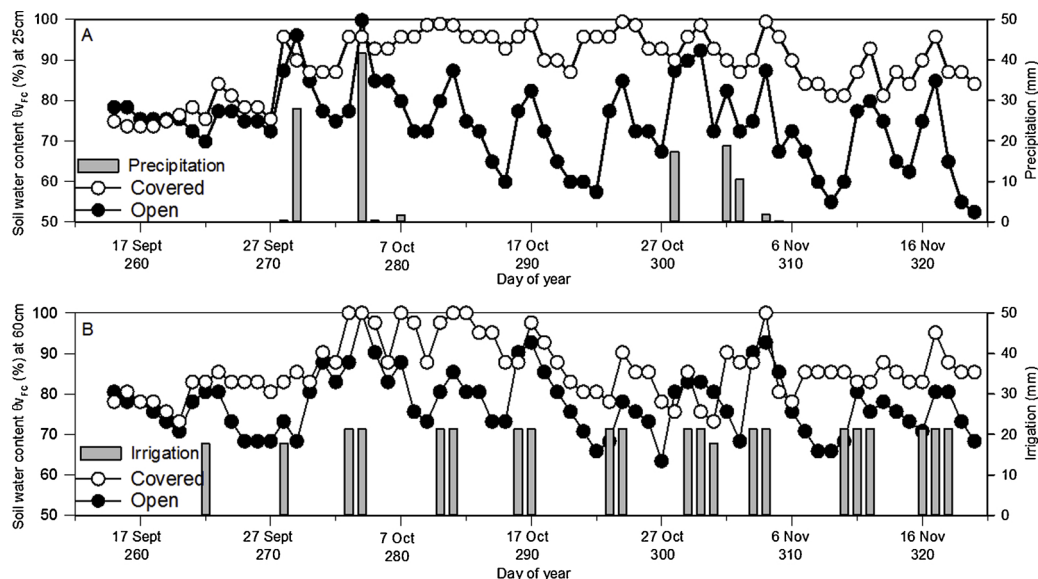
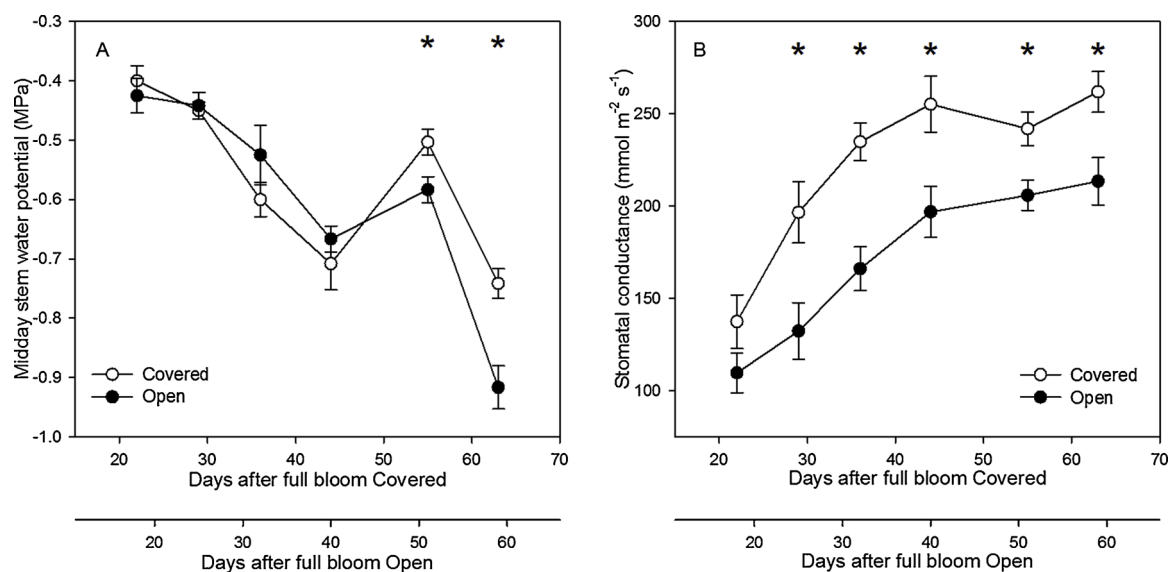


Fig. 2. (A) Seasonal volumetric water content refers to field capacity ( $\theta_{vFC}$ , %) daily mean value at 25 cm depth and daily rainfall (mm) and (B)  $\theta_{vFC}$  at 60 cm depth and volume of water applied (mm). Each data point represents the mean of three blocks (replications).



**Fig. 3.** (A) The seasonal course of midday stem water potential ( $\Psi_{stem}$ ) and (B) stomatal conductance ( $g_s$ ) in 'Royal Dawn' on 'MaxMa 14' sweet cherries under cover and in the open (control). Each data point represents the mean  $\pm$  SE ( $n = 5$ ). Values marked \* are significantly different ( $p < 0.05$ ) based on ANOVA. Harvest occurred on 56 DAFB under cover and 60 DAFB in the open.

development but the values under cover were generally 30% higher than in the open (Fig. 3B).

Full bloom occurred four days earlier under cover (on DOY 254). The lengths of Stages I, II and III of fruit development were similar between the two treatments and followed a typical double-sigmoid pattern. The fruit development period was shorter 56 d (636 GDD, growing degree days, base 4 °C) under cover and longer 60 d (609 GDD) in the open (Table 1). In addition, covered trees were harvested 8 days earlier (DOY 310) than open field trees (DOY 318).

At commercial harvest, fruit diameter (mm) and weight (g) were significantly higher in fruit from under the covers. There were no significant differences in fruit size among the three canopy layers (bottom, middle and top) either under cover or in the open (Fig. 4A and B). However, the use of covers influenced the initial absolute fruit growth rate (AGR) (Fig. 4C). Fruit under cover showed significantly higher AGR values between 14 and 44 DAFB. The AGR values declined in both treatments but fruit from the trees in the open showed higher AGRs in late Stage III.

### 3.3. Leaf area

Vegetative growth at harvest did not show significant differences between treatments (Table 2). However, it followed a different pattern under cover and in the open. Spur leaves grew faster under cover. Spur leaf areas stabilized at values greater than 40 cm<sup>2</sup> two weeks earlier under cover (DOY 284) than in the open (DOY 299). Extension shoots increased in length faster under cover than in the open, with significant

differences between treatments during the first days of measurement but without significant differences at harvest. Extension shoot length and leaf number did not differ between treatments (Table 2). Spur leaves contributed 63% of total LA. There were no significant differences between treatments in the number of spurs per tree, nor in the number of leaves per spur (Table 2).

There were no significant differences in total LA, fruit number per tree or LA/F ratios between treatments (Table 3). However, trees under cover tended to higher values of LA and, especially, for LA per extension shoot, than in the open.

### 3.4. Fruit development, yield and fruit quality

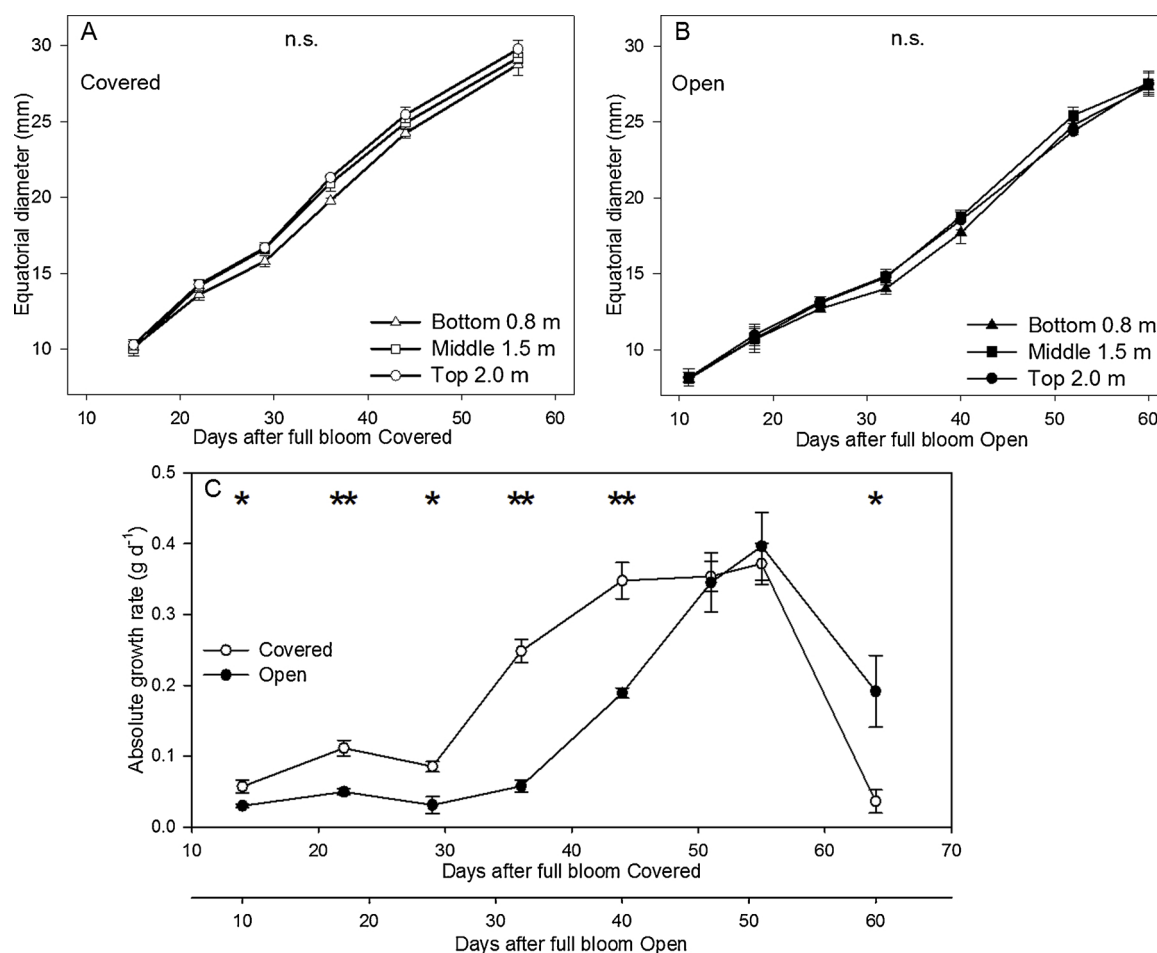
Sweet cherry trees grown under cover were harvested at 56 DAFB (DOY 310), 8 d earlier than trees in the open (Table 1). There was significant interaction between canopy layer (bottom, middle, top) and treatment (i.e. covered, open) (Table 4). Although no significant differences in total yield (values in the range 14 to 16 kg/tree) were observed between treatments (Fig. 5A), yield varied with treatment among the canopy layers (Table 4). In both treatments, the middle layer of the canopy (1.5 m) was significantly more productive than either the bottom or top layers.

The middle canopy layer produced 51% of the total yield per tree (8.3 kg tree<sup>-1</sup>) under cover and 46% (6.5 kg tree<sup>-1</sup>) in the open (Fig. 5A, Table 5). There were no significant differences in fruit quality parameters within a treatment, between the different canopy layers (Table 4).

**Table 1**

Lengths of fruit development stages as day of the year (DOY), days after full bloom (DAFB) and growing degree days base 4 °C (GDD) in sweet cherry 'Royal Dawn' on 'MaxMa 14', under cover and in the open.

Developmental Stage	Covered				Open			
	DOY	DAFB	Duration (d)	GDD	DOY	DAFB	Duration (d)	GDD
Full Bloom	254	0	6	60.0	258	0	8	61.3
Fruit Set	260	6	4	56.2	265	8	5	53.0
Stage I	264	10	13	135.6	271	13	14	116.2
Stage II	277	23	7	67.8	285	27	6	59.7
Stage III	284	30	26	316.8	291	33	27	318.9
Harvest	310	56			318	60		



**Fig. 4.** Seasonal patterns of fruit diameter in the three canopy layers 0.8 m (bottom), 1.5 m (middle) and 2.0 m (top) of ‘Royal Dawn’ on ‘MaxMa 14’ sweet cherry trees (A) under cover and (B) in the open (control). n.s. indicates no significant differences between treatments based on ANOVA. (C) Pattern of absolute growth rate of sweet cherry fruit from the top (2 m) canopy layer. Each data point represents the mean ± SE (n = 5). Values marked \* and \*\* are significantly different at p < 0.05 and p < 0.01, respectively. Harvest for covered trees was on 56 DAFB and for trees in the open was on 60 DAFB.

**Table 2**

Spur and extension shoot at harvest of total leaf area (LA, cm<sup>2</sup>) of sweet cherry ‘Royal Dawn’ on ‘MaxMa 14’ under cover (DOY 310) and in the open (DOY 318).

		Spurs			Extension shoots		
		Spur number	Leaf number	LA spur (cm <sup>2</sup> )	Shoot number	Leaf number	LA shoot (cm <sup>2</sup> )
<b>Covered</b>	Mean	517.4	8.4	42.9	107	24.2	51.1
	S.E.	39.6	0.5	0.6	5.7	1.4	2.1
<b>Open</b>	Mean	480.8	9.2	41.7	93	20.8	47.0
	S.E.	36.9	0.7	0.8	6.9	2.2	2.3
ANOVA	p-value	0.518	0.381	0.262	0.147	0.240	0.228

**Table 3**

Total leaf area at harvest (LA, m<sup>2</sup>) per tree, total number of fruit (F, n) per tree and leaf area per fruit (LA/F, cm<sup>2</sup> fruit<sup>-1</sup>) for sweet cherry ‘Royal Dawn’ on ‘MaxMa 14’ grown under cover (DOY 310) and in the open (DOY 318).

		Total LA tree <sup>-1</sup>	n <sup>o</sup> fruit tree <sup>-1</sup>	LA/F
		(m <sup>2</sup> )		(cm <sup>2</sup> fruit <sup>-1</sup> )
<b>Covered</b>	Mean	31.6	1609	199.5
	S.E.	2.0	106	18.3
<b>Open</b>	Mean	28.0	1552	185.7
	S.E.	1.6	123	23.2
ANOVA	p-value	0.222	0.770	0.544

The use of covers had a significant effect on fruit quality. Compared to fruit from trees in the open, average fruit size under the covers was 10% higher but showed lower SSC and firmness (Table 4). Average fruit size was 10% higher under cover (Table 4) and 67% of the fruit from trees under cover was of diameter 28 to 32 mm or more (Fig. 5B, Table 5). No double fruits were observed in either treatment.

### 3.5. Cracking susceptibility

Trees under covers showed significantly lower cracking losses than trees in the open. At harvest, fruit from the trees in the open were significantly more cracked (19%) than those from the covers (3%) (Tables 4 and 6). Styler-end cracks predominated under the covers, and none were > 2 mm (data not shown). The fruit on the trees in the open exhibited stem - end cracking (37%), cheek cracking (16%) and styler - end cracking (47%). Also in the open, there were significant differences in cracking in the different canopy layers (Table 6). Fruit from the top layer were significantly more cracked than from the bottom layer (Table 6).

Fruit from trees in the open showed a higher potential for cracking as evaluated by CI index. This was particularly evident for fruit immersed in the acid (pH 4) medium (Table 6). Under these conditions, fruit from the covered trees and in the top canopy layer had a higher CI than fruit from the bottom layer. In general, CI was higher for fruit from trees in the open than from the covered trees (Table 6).

**Table 4**  
Effect of canopy layer (bottom 0.8 m, middle 1.5 m, top 2.0 m) and treatment (covered, open) on yield and quality parameters of sweet cherry ‘Royal Dawn’ on ‘MaxMa 14’.

		Total yield (kg tree <sup>-1</sup> )	N° fruits per tree	Cracked fruits (%)	Unitary weight (g)	Diameter (mm)	SSC (%)	TA (g L <sup>-1</sup> )	Firmness (Durofel units)
Layer of canopy	Top	4.0b	418b	14.3a	9.5a	28.6a	19.0a	9.8a	74.2a
	Middle	7.4a	765a	10.7a	9.7a	28.4a	18.8a	9.8a	75.4a
	Bottom	3.7b	390b	7.6a	9.5a	28.0a	17.9a	8.9a	74.4a
Treatment	Covered	16.1a	1609a	3.1b	10.0a	29.2a	16.7b	10.0a	72.0b
	Open	14.1a	1552a	18.7a	9.1b	27.5b	20.3a	9.1a	77.3a
ANOVA		p value							
A	Layer	< 0.001	< 0.001	0.305	0.836	0.546	0.490	0.284	0.716
B	Treatment	0.386	0.757	< 0.001	< 0.001	< 0.001	< 0.001	0.088	< 0.001
A x B	Interaction	0.009	0.060	0.142	0.539	0.478	0.877	0.974	0.348

Canopy layer values are means over the ten blocks. Treatment values are the means over the five blocks of each treatment. Different letters in the same column indicate significant differences between tree layers in the same canopy (A) based on Duncan’s test ( $p < 0.05$ ) or among treatments (B) based on ANOVA ( $p < 0.05$ ).

#### 4. Discussion

##### 4.1. Environmental monitoring

As expected, the use of high tunnels modified environmental conditions during the period of fruit development in the combination ‘Royal Dawn’ on ‘MaxMa 14’. Compared to the open, the covers increased air temperatures by 5 to 10 °C during the period of fruit development (Fig. 1A.1). Several studies (Black and Drost, 2010; Waterer and Bantle, 2000; Wien, 2009) have reported temperature differences of 10 to 15 °C between inside and outside tunnels at solar midday. High tunnels also increased minimum air temperatures by 1 to 3 °C. This effect was more evident on cooler days (Fig. 1A.2).

We observed no negative effects of either low or high air temperatures under the covers. Similar commercial yields between covered and uncovered trees suggest optimal pollination, fruit set and subsequent fruit development inside the high tunnels. Mean air temperatures under the covers between bloom and fruit set averaged 13.9 °C. This is 4.3 °C higher than in the open. This is important since pollination of sweet cherry is highly sensitive to warm (27 – 30 °C) temperatures, due to loss of stigmatic receptivity and reduction of the effective length of the pollination period (Garcia-Montiel et al., 2010). In sweet cherry ‘Vignola’, ‘Sunburst’, ‘Napoleon’ and ‘Burlat’ grafted onto SL64, pollen germination took between 8 d and 2 d at temperatures between 10 and 30 °C, respectively (Hedhly et al., 2003).

The higher temperatures inside the high tunnels during fruit development may have speeded cell division and cell expansion (Bastías et al., 2014; Retamal-Salgado et al., 2015) since harvest was 8 d earlier in the covered trees. On the other hand, minimum air temperatures

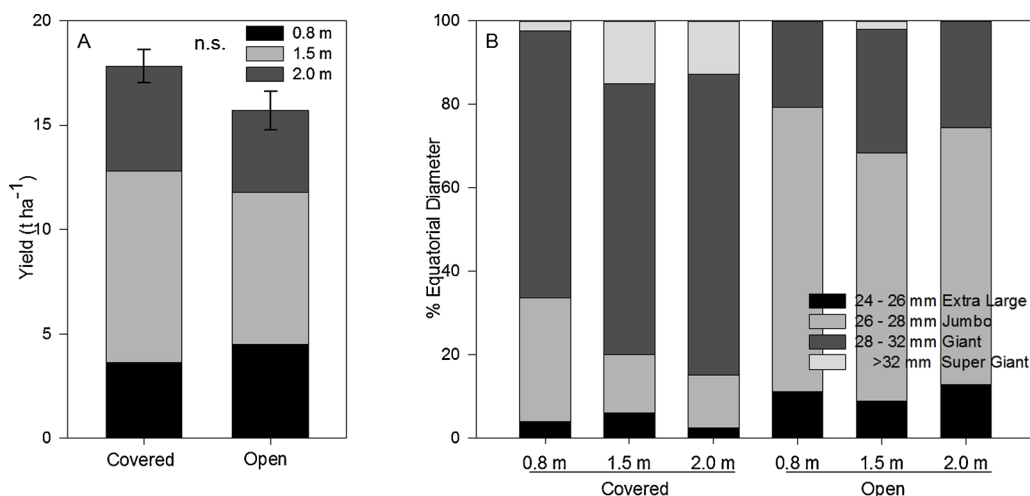
under the covers between bloom and fruit set averaged 6.6 °C, and there were no frost events. These results agree with Lang (2009), who reported tunnels as an effective way to protect sweet cherry against cold.

Trees under high tunnels received 20% lower irrigation than uncovered trees. These results are in agreement with Lamont (2005), who reported more efficient water use in protected crops. The covered trees showed higher  $\theta_{vFC}$  due to the protected environment, which reduced evapotranspiration. Accordingly, soil water content under the covers was more stable (Fig. 2). Conservation of soil moisture under tunnels has been reported previously (Montri and Biernbaum, 2009). The total yield of covered and open trees was similar (Table 3); however the fruit-size distribution in the open trees was concentrated between 26 and 28 mm (Jumbo), while that in the covered trees was between 28 and 32 mm (Giant). It is likely a positive effect of an optimal water supply under the tunnels during Stage III (Fig. 5B).

Slightly higher RH values measured inside high tunnels (Fig. 1B). High values of RH and VPD (but not excessive, i.e. below 3 or 4 kPa), with good soil water availability, have been related to increases in stomatal conductance in sour cherry (*Prunus cerasus* L; Flore, 1985) and ‘Prime Giant’/‘SL64’ sweet cherry (Blanco et al., 2018). According to Brüggewirth et al. (2016), the difference in water vapor concentration between the fruit and the environment drives water through the plant and promotes fruit growth in the combination ‘Sam’/‘Gisela 5’.

##### 4.2. Tree measurements

There were no significant differences between treatments in  $\Psi_{stem}$  during fruit development (Stages I, II and early III). At harvest, open trees showed  $\Psi_{stem}$  values below -0.90 MPa (Fig. 3A), while  $\Psi_{stem}$



**Fig. 5.** (A) Harvest yield distribution within each treatment for each canopy layer and (B) fruit equatorial diameter distribution ( $n = 125$ ) for each treatment and with canopy layer in ‘Royal Dawn’ on ‘MaxMa 14’ sweet cherry trees under covers and in the open. In (A) each bar represents the mean  $\pm$  SE ( $n = 5$ ). n.s. indicates differences were not significant ( $p < 0.05$ ) based on ANOVA.

**Table 5**

Fruit yield and quality parameters of sweet cherry 'Royal Dawn' on 'Maxma 14' from the bottom (0.8 m), middle (1.5 m) and top (2.0 m) canopy layers of the covered trees and those in the open. SSC, soluble solids concentration; TA titratable acidity; MI, maturity index.

		Total yield (kg tree <sup>-1</sup> )	Unitary weight (g)	Equat. Diam. (mm)	Polar Diam (mm)	SSC (%)	TA (g L <sup>-1</sup> )	MI	Firmness (Durofel)
<b>Covered</b>	Top	4.5b	10.1	29.8	23.0	17.1	10.3	1.7	70.8
	Middle	8.3a	10.0	29.2	22.8	17.1	10.2	1.2	72.8
	Bottom	3.3c	10.0	28.8	22.6	16.1	9.4	1.8	72.4
	ANOVA	< 0.001	0.679	0.288	0.381	0.077	0.536	0.852	0.262
	p-value								
<b>Open</b>	Top	3.6b	8.9	27.5	20.9	20.9	9.3	2.3	77.6
	Middle	6.4a	9.3	27.5	21.3	20.5	9.4	2.2	78.0
	Bottom	4.1b	9.0	27.3	21.2	19.7	8.5	2.3	76.4
	ANOVA	< 0.001	0.475	0.924	0.507	0.277	0.519	0.654	0.531
	p-value								

All values are the means of the five blocks. Different letters in the same column indicate significant differences between canopy layers within a treatment based on Duncan's test ( $p < 0.05$ ).

**Table 6**

Fruit cracking incidence (%) at harvest in sweet cherry 'Royal Dawn' on 'Maxma 14'. Cracking index (CI) after fruit had been immersed in tap water not-adjusted for pH (pH 7) or under an acid solution (pH 4). Fruit were taken from the bottom (0.8 m) or top (2.0 m) layers of the canopy of trees both under cover and in the open.

		Cracking at harvest %	CI in laboratory	
			pH 7	pH 4
<b>Covered</b>	Top	4.4	41.1	51.7
	Bottom	2.0	32.3	40.5
<b>Open</b>	Top	24.2	52.8	68.8
	Bottom	13.2	52.3	67.5
ANOVA	p-value			
A	Covered	0.383	0.169	0.198
B	Open	0.036	0.924	0.873
	Top	< 0.001	0.017	0.120
	Bottom	0.002	0.015	< 0.001

All values are means of the five blocks. p-value for covered and bottom are the analyses of variance for the different canopy layers (top and bottom) within the same treatment. p-value for top and bottom are the analysis of variance for each layer, top and bottom, comparing trees covered and in the open. Cracking index (CI) was calculated with 50 fruits as:  $CI = (5a + 3b + c) / (250)$ , where: a, b and c were the numbers of cracked fruit at 2, 4 and 6 h after immersion, respectively. The immersion solutions were tap water (pH 7) and an acidic medium (pH 4).

values of covered trees were higher (-0.50 MPa). Marsal et al. (2009) reported -0.9 MPa as a limit for well-irrigated mature 'New Star'/'SL64' sweet cherry trees. In addition, trees under the covers always showed higher  $g_s$  values than trees in the open. At harvest, covered trees and those in the open showed  $g_s$  values above 200 mmol m<sup>-2</sup> s<sup>-1</sup> (Fig. 3B). Similar values have been reported for the combination 'Summit'/'SL64' by Marsal et al. (2010). Higher  $g_s$  values under the covers may indicate higher gas exchange capacity, including higher CO<sub>2</sub> assimilation and transpiration (Retamal-Salgado et al., 2015).  $\Psi_{stem}$  and  $g_s$  values, were highly dependent on VPD and soil water content in 'Prime Giant'/'SL64' sweet cherry (Blanco et al., 2018), as well as in other deciduous fruit trees (De la Rosa et al., 2013; Intrigliolo and Castel, 2005; Pérez-Pastor et al., 2009). In our study, covered trees showed higher values of soil water content,  $\Psi_{stem}$  and  $g_s$ , suggesting a beneficial effect on fruit size.

#### 4.3. Leaf area

In sweet cherry, Lang (2014) reported that 'Early Robin'/'Gisela 12' trees under cover developed higher total LAs than trees in the open. However, in our study, the high tunnels had no effect total LA at harvest, but LA did develop faster under cover. This may in part explain the early harvest and larger fruit. Source limitation due to low LA

development early in the growing season may affect fruit cell division if storage reserves are depleted (Ayala, 2004; Flore and Layne, 1999; Olmstead et al., 2007). This effect may be particularly important in highly productive early cultivars such as 'Royal Dawn'.

In addition, there were no significant differences between the covered and open trees in LA/F ratio, where LA ranged between 186 and 200 cm<sup>2</sup> per fruit (Table 3). An adequate LA/F ratio ensures availability of photoassimilate to obtain high quality fruit (Ayala and Lang, 2015; Ayala and Lang, 2017; Koumanov et al., 2018; Villasanté et al., 2012). In this study, LA/F ratios were higher than the optimum of 99 cm<sup>2</sup> per fruit proposed by Usenik et al. (2010) for the mid-season cultivars 'Lapins'/'Gisela 5', but lower than proposed by Whiting and Lang (2004) 244 cm<sup>2</sup> per fruit for another mid-season cultivar 'Bing'/'Gisela 5'. Some degree of carbon limitation (Ayala, 2004; Ayala and Lang, 2017) may have occurred in the open trees, which bore smaller fruit (9 g, 27.5 mm diam) than the covered ones (10 g, 29 mm diam). Although the uncovered trees produced smaller fruit, it was still good enough to export, suggesting that a LA/F ratio of 193 cm<sup>2</sup> fruit<sup>-1</sup> is adequate for the combination 'Royal Dawn'/'Maxma 14'.

#### 4.4. Fruit development, yield and quality

The use of covers shortened the duration of fruit development and allowed a slightly earlier harvest. Covered 'Royal Dawn'/'MaxMa 14' trees were harvested 8 d earlier than the trees in the open. Fruit development followed a double-sigmoid pattern (Coombe, 1976) in covered and open sweet cherry trees but the period of fruit growth was 4 d shorter under the tunnels (Fig. 4 A and B). Although the length of the various phenological stages of fruit development is genetically regulated (Fadon et al., 2015; Olmstead et al., 2007), the use of high tunnels allowed more rapid GDD accumulation (Table 1).

Fruit size, SSC, firmness and color are the important consumer attributes for sweet cherry (Crisosto et al., 2003) and commercial harvest is usually on the basis of these (Serrano et al., 2005). Trees under covers showed similar yields to those in the open (Fig. 5A). Regardless of position in the canopy (layer - bottom, middle, top) the fruit from the covered trees was larger but had lower SSC (17%) and firmness (7%) than fruit from open trees (Table 4). These results agree with Lang (2009; 2014) who indicated higher unitary fruit weight of 'Rainier'/'Gisela 6' and 'Rainier'/'Gisela 5' inside tunnels. The combination of larger fruit and lower SSC has been reported previously for covered trees of sweet cherry 'Sweetheart'/'Colt' throughout fruit development (Meland et al., 2017). This may be explained by the lower light levels under a cover. However, the lower SSC under the covers could be due to dilution by the high water content and large fruit size as was reported for the combinations 'Summit'/'SL64' and 'Prime Giant'/'SL64' under water stress by Marsal et al. (2010) and Blanco et al. (2019). Regarding fruit firmness, fruit of covered and open trees resulted in firmness > 70



Durofel units, which for 'Royal Dawn', is suitable for export to distant markets. Fruit firmness is important not only for consumer acceptance but also for storage (Hampson et al., 2014; Martínez-Romero et al., 2006; Zoffoli et al., 2017). Our results agree with those of Meland et al. (2017) who found that with covered 'Sweetheart'/'Colt' sweet cherries, from bloom to harvest, were less firm than those were left open.

#### 4.5. Cracking susceptibility

In sweet cherry, cracking susceptibility increases as fruit develops (Balbotín et al., 2014) and varies among cultivars (Lane et al., 2000; Measham et al., 2009; Quero-García et al., 2017; Stojanović et al., 2013). In our study a 40 mm rain event occurred at 23 DAFB (during Stage II) in the covered trees and at 19 DAFB (during Stage I) in the open trees but as expected, no cracking was observed in either treatment. However, later on at 13 d before harvest of the open trees (during Stage III) rain over two days (29 mm rainfall) caused 19% cracking in the open trees. However, inside the high tunnel the incidence of cracking was only 3%. Cline et al. (1995) reported 5% of cracked fruit in covered 'Ulster'/'Colt' sweet cherry trees and related it to the susceptibility of the combination to the influx of water through the pedicel. Later, Lang (2014) determined that covering systems, such as tunnels, exclude rainwater contact with the fruit, eliminating stem-end cracking in 'Rainier'/'Gisela 5' and 'Rainier'/'Gisela 6'.

The laboratory evaluation of cracking susceptibility found that fruit from trees in the open had a higher CI than that from the covered trees. Healthy fruit from the trees in the open were of higher cracking susceptibility than that from the covered trees (Table 4). Peschel and Knoche (2005) related high cracking susceptibility in sweet cherry 'Hedelfinger', 'Kordia', 'Sam' and 'Van', all grafted on 'Alkavo', with the presence of microcracks. The difference in CI between covered and uncovered trees may be related to the presence of more microcracks on fruit from the open. It is likely that microcracks predispose sweet cherry fruit to macroscopic cracking, particularly at the styler end. On trees in the open, the fruit is exposed to direct rainfall, sunlight, wind and more variable levels of soil water content. These more rigorous environmental conditions may expose the cuticular membrane to increased strains and stresses during development leading to a higher CI. Our results have important postharvest implications since fruit that is more sensitive to crack could develop diseases during storage (Martínez-Romero et al., 2006; Zoffoli et al., 2017). Fruit on covered trees showed lower CI values than fruit from the open, suggesting likely better performance during storage. Postharvest cracking has been reported as a result of long exposure of fruit to water during hydrocooling or due to absorption of condensed moisture (Wani et al., 2014; Wang and Long, 2015). Chilean sweet cherries are exported mainly to China by boat using saturated atmospheres and modified atmosphere packaging, which is prone to condensation. This is particularly important in 'Royal Dawn', which is unsuited to distant markets due to its high cracking sensitivity when exposed to modified atmosphere packaging. Accordingly, the production of 'Royal Dawn' under high tunnel may reduce fruit stress and so allow better postharvest performance.

#### 5. Conclusions

This study confirms that high tunnels offer protection against rainfall and reduces postharvest cracking potential, which is key for export to distant markets. As expected, the use of high tunnels reduced rain-induced fruit cracking in 'Royal Dawn'.

High tunnels stopped rain reaching the fruit surface and increased air temperatures compared to the open. Trees under high tunnels received 20% lower irrigation than open trees. Higher air temperatures under the covers speeded fruit growth and brought forward the dates of bloom and fruit development. As a direct consequence, covered trees were harvested 8 d earlier than trees in the open. There were no detectable differences either in yield, vegetative growth and in the LA/F

ratio between covered and open trees. Fruit under the covers was 10% larger but it had lower SSC and firmness.

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