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Response of some emergent crops (persimmon, pomegranate and quince) to water deficit

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TESIS DOCTORAL

Isabel Griñán Mora
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Response of some emergent crops (persimmon, pomegranate and quince) to water deficit

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Thesis for the degree of Doctor from the
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Response of some emergent crops (persimmon, pomegranate and quince) to water deficit

Thesis presented by Isabel Griñán Mora to qualify for Doctor degree from
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This doctoral thesis corresponds to a **compendium of publications category** selected to qualify for **Doctor** degree from Miguel Hernández University of Elche.

To this end, the **selected papers** and the quality, in accordance with the **2017** edition of **Journal Citation Reports**®, of each one are indicated as follows:

- Galindo[1], A., Calín-Sánchez[1], A., **Griñán[1], I.,** Rodríguez, P., Cruz, Z.N., Girón, I.F., Corell, M., Martínez-Font, R., Moriana, A., Carbonell-Barrachina, A.A., Torrecillas, A., Hernández, F. 2017. Water stress at the end of pomegranate fruit ripening permits earlier harvesting and improves fruit quality. *Scientia Horticulturae* 226: 68-74. DOI: *10.1016/j.scienta.2017.08.029*

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CENTRO DE EDAFOLOGÍA Y BIOLOGÍA
APLICADA DEL SEGURA

Arturo Torrecillas Melendreras, Profesor de Investigación del CSIC y Alejandro Galindo Egea, Investigador Postdoctoral en la Universidad de Twente

Informan

Que la Tesis Doctoral ***Response of some emergent crops (persimmon, pomegranate and quince) to water deficit***, de la que es autora la Master en Ingeniería Agronómica **Isabel Griñán Mora**, ha sido realizada bajo nuestra dirección y supervisión en el Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC).

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En Murcia, a 5 de Marzo de 2019

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CERTIFICA:

Que ha tenido acceso a los contenidos de la Tesis Doctoral titulada ***Response of some emergent crops (persimmon, pomegranate and quince) to water deficit*****,** de la que es autora la Master en Ingeniería Agronómica **Isabel Griñán Mora,** actuando como director el Dr. **Arturo Torrecillas Melendreras,** del CEBAS-CSIC**,** codirector el Dr. **Alejandro Galindo Egea**, de la Universidad de Twente, y tutor el Dr. **Federico Ferreres de Arce,** del CEBAS-CSIC.

En consecuencia de todo ello, considera que tanto el contenido como la forma se adecuan a los requerimientos del Programa de Doctorado ReTos-AAA y, por tanto, es apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos, firmo el presente certificado en Orihuela a seis de Marzo de dos mil diecinueve.

Dr. Ángel A. Carbonell Barrachina
Coordinador Programa Doctorado ReTos-AAA

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1.Doctoral thesis structure

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Structure

The content of this thesis has been prepared in agreement with the internal regulations of the Miguel Hernández University of Elche for the presentation of **Doctoral Thesis as a compendium of publications**. So, this thesis presents structure as follows:

- **Abbreviations and symbols**, where the abbreviations and symbols used in the work are explained.
- **Abstract and Resumen,** where the most significant results and conclusions are exposed.
- **Introduction,** where it is studied the state of the art on the response of persimmon, quince and pomegranate trees to deficit irrigation, paying special attention to the mechanisms developed by each crop to confront drought and the effects of water deficit on yield and fruit quality.
- **Objectives**, where the global and partial objectives of the research are detailed.
- **Material and methods**, where the agronomic characteristics of the experimental plots are indicated, as well as all the methodologies to determine the plant water status and the physical and chemical characteristics of the persimmon, pomegranate and quince fruits.
- **Publications**, where other six papers are included (five published SCI papers and a complementary paper pending of journal approval)).

  – In the first publication (*Agricultural Water Management* 202: 311-324 (2018). DOI: 10.1016/j.agwat.2017.08.015, Open Access) the state of the art on the deficit irrigation strategies and the response of some emerging crops was reviewed. Then, the factors involved in establishing an identity brand (*hydroSOS*) to protect fruits obtained under specific deficit irrigation conditions are discussed.

  – In the second publication (*Agricultural Water Management,* 217, 391-398 (2019). DOI *10.1016/j.agwat.2019.03.008, Open Access*) it is presented the sensitivity of plant water status indicators to be used in irrigation scheduling in persimmon trees and the mechanisms developed by this crop in response to mild water stress.

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  - In the third publication (*Agricultural Water Management* 221, 66-72 (2019). DOI*: 10.1016/j.agwat.2019.04.017, Open Acces)* it is showed the mechanisms developed by quince trees at leaf level to confront water stress.
  - The fourth publication (*Scientia Horticulturae* 244: 68-74 (2019). DOI: *10.1016/j.scienta.2018.09.013*), shows the effect of withholding irrigation water in the middle of the rapid fruit growth period on yield, main fruit physicochemical characteristics, sensory profile, and volatile composition of quince (cv. BA-29).
  - The fifth publication (*Scientia Horticulturae* 226: 68-74 (2017). DOI: *10.1016/j.scienta.2017.08.029*) studies the response of pomegranate fruit to drought stress during the phenological period of fruit ripening. Special attention was paid to the effects on plant productivity of water restrictions during fruit ripening and whether such restrictions have secondary effects on fruit characteristics and composition
  - The sixth publication (*Journal of the Science of Food and Agriculture* 99, 1425-1433 (2019). DOI: *10.1002/jsfa.9324*, *Open Access*) shows the interaction between preharvest pomegranate fruit bagging and plant water status on the sensory and quality attributes of the fruit. In addition, the effect of both factors on fruit sunburn and fruit splitting was studied as a complementary objective
  - The seventh publication (*Scientia Horticulturae* 247: 247-253 (2019). *DOI: 10.1016/j.scienta.2018.12.017*) studied the effect of chitosan spraying in fully irrigated and water stressed trees on yield, fruit quality and the occurrence of fruit peel physiopathies.

- **Results and Discussion**, where the most interesting and important results are analyzed and discussed.
- **Conclusions**, where the final conclusions are listed.
- **References**, where the references used in sections complementary to the publications are indicated.

2. Abbreviations and symbols

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Abbreviations

A	Absorbance
$a*$	Red-greenness colour
AA	Antioxidant activity
AW	Arils weight
ABA	Abscisic acid
$\mathrm{ABTS}^{\cdot+}$	(2,2-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation
ANOVA	Analysis of variance
$b*$	Blue-yellowness colour
B	Bagging fruits
$C*$	Chroma
CA	Citric acid
C3G	Cyaniding-3-glucoside
DF	Dilution factor
DHFI	Deficit high-frequency irrigation
DI	Deficit irrigation
DOY	Day of the year
DPPH	Radical 2,2-diphenyl1-picrylhydrazyl method
DVB/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
ED	Equatorial diameter
ETc	Crop evapotranspiration
ETo	Crop reference evapotranspiration

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FDR	Frequency Domain Reflectometry
FI	Full Irrigation
FIB	Full irrigated and bagged fruits
FINB	Full irrigated and non-bagged fruits
FINQ	Full irrigated and non-sprayed with Quitomax® fruits
FIQ	Full irrigated and sprayed with Quitomax® fruits
FL	Fruit length from calyx to base
FRAP	Ferric-reducing antioxidant power
Fru	Fructose
FW	Fruit weight
GAE	Gallic acid equivalents
GLM	General linear model
H^o	Hue angle
Glu	Glucose
HPLC	High performance liquid chromatography
HS-SPME	Headspace solid phase micro-extraction
L^*	Lightness
LSD	Fisher's Least Significant Difference
LVDTs	Linear variable displacement transducers
MA	Molar absorptivity of cyaniding-3-glucoside
MDS	Maximum daily trunk shrinkage
MI	Maturity index
MW	Molecular weight

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MY	Marketable yield
NB	Non-bagged fruits
NQ	Fruits non-sprayed with Quitomax®
Pn	Net photosynthesis
PT	Peel thickness
PV	Pressure-volume
PRD	Partial root-zone drying
PTFE	Polytetrafluoroethylene
Q	Fruits sprayed with Quitomax®
RDI	Regulated deficit irrigation
RH	Relative humidity
RWC	Relative leaf water content
RWC_a	Relative leaf apoplastic water content
RWC_o	Relative leaf water content at full turgor
RWC_{tlp}	Relative leaf water content at the turgor loss point
SA	Succinic acid
SDI	Sustained deficit irrigation
SF	Sap flow
SI_{gleaf}	Signal intensity of leaf conductance
$SI_{\Psi fruit}$	Signal intensity of midday fruit water potential
$SI_{\Psi leaf}$	Signal intensity of leaf water potential
$SI_{\Psi stem}$	Signal intensity of midday stem water potential
SPI	Splitting

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SUI	Sunburn
TA	Titratable acidity
TAC	Total anthocyanin content
TAA	Total antioxidant activity
TDF	Daily trunk diameter fluctuations
Tm	Daily mean air temperature
TPC	Total phenolic content
TSS	Total soluble solids
TY	Total yield
USFDA	Administración de alimentos y medicamentos de Estados Unidos
VPDm	Daily air vapor pressure deficit
WUE	Water use efficiency
WP	Water productivity
WS	Water stress
WSB	Water stressed and bagged fruits
WSNB	Water stressed and non-bagged fruits
WSNQ	Water stressed and non-sprayed with Quitomax® fruits
WSQ	Water stressed and sprayed with Quitomax® fruits

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Symbols

Є	Leaf bulk modulus of elasticity
Λ	Intercept of the relationship
σ	Slope
θ_v	Soil volumetric water contents
Ψ_{fruit}	Midday fruit water potential
Ψ_L	Minimum leaf water potential
Ψ_{leaf}	Leaf water potential
Ψ_{md}	Midday leaf water potential
Ψ_{os}	Leaf osmotic potential at full turgor
Ψ_p	Leaf turgor potential
Ψ_{pd}	Predawn leaf water potential
Ψ_{pmd}	Midday leaf turgor potential
Ψ_{ppd}	Predawn leaf turgor potential
Ψ_s	Soil water potential
Ψ_{smd}	Midday leaf osmotic potential
Ψ_{spd}	Predawn leaf osmotic potential
Ψ_{stem}	Midday stem water potential
Ψ_{stlp}	Osmotic potential at the turgor loss point
Ψ_{tlp}	Leaf water potential at the turgor loss point

g_{leaf}	Leaf conductance
g_{lmd}	Midday leaf conductance

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3. Resumen and Abstract

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Resumen

El presente estudio tuvo como objetivo profundizar en el conocimiento de la respuesta de tres cultivos emergentes, caqui (*Diospyros kaki*), granado (*Punica granatum*) y membrillero (*Cydonia oblonga*), al estrés hídrico y desarrollar estrategias para mejorar el ahorro de agua y la calidad de los frutos. Para complementar el estado actual de conocimientos, se desarrollaron distintos ensayos en los mencionados cultivos.

En primer lugar se estudiaron los mecanismos de resistencia (evitación y tolerancia) desarrollados por plantas jóvenes de caqui ‘Rojo brillante’ en respuesta al déficit hídrico suave y la sensibilidad para diagnosticarlo de los indicadores del estado hídrico más usuales, medidos tanto de forma continua como discontinua. Los resultados indicaron que esta especie desarrolla un gradual control estomático (mecanismo de evitación del estrés) y exhibió algunos caracteres xeromórficos tales como altos contenidos de agua apoplástica, que podría facilitar la retención de agua a bajos potenciales hídricos. Las tasas de flujo de savia resultaron ser el indicador más adecuado para detectar cambios en el estado hídrico del cultivo, ya que presentaron los valores más altos de la relación intensidad de señal:ruido y son capaces de informar de forma continua y en tiempo real del estado hídrico.

Igualmente, en plantas de membrillero BA-29 adultas se estudiaron los mecanismos de resistencia al déficit hídrico. Las plantas exhibieron un comportamiento extremadamente anysohídrico en respuesta al estrés hídrico. Además, durante el desarrollo del estrés, se produjo un ajuste osmótico activo que contribuyó al mantenimiento de altos niveles de conductancia foliar y, por tanto, de productividad. Los bajos niveles de agua apoplástica de este cultivo pueden favorecer un mayor gradiente de potencial hídrico entre el suelo y la hoja, lo que favorecería la absorción de agua. Por otra parte, la fase de rápido crecimiento del fruto resultó claramente crítica ante el estrés hídrico, ya que los frutos en esas

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condiciones disminuyeron su peso unitario, tamaño y contenido de humedad, lo que se tradujo en una significativa disminución de la producción comercial. Sin embargo, los membrillos en estas condiciones presentaron un mayor contenido en sólidos solubles y polifenoles, aunque la actividad antioxidante disminuyó. El aumento de la coloración de la piel y la mejora de algunas características esenciales de la pulpa (disminución de la fibrosidad y acorchado y aumento del carácter crujiente) son aspectos de alto interés para la comercialización de la fruta.

En los estudios enfocados a optimizar el momento de recolección de la granada Mollar de Elche de plantas autoenraizadas, se confirmó que el periodo de maduración es claramente crítico ya que el riego resultó imprescindible para alcanzar máximas producciones. No obstante, un periodo muy corto de supresión del riego (6 días) al final de la maduración implica ahorro de agua, adelanto de la maduración y un aumento del contenido en sustancia bioactivas (antocianos, compuestos fenólicos, punicalaginas y ácido elágico) de los frutos sin modificar el tamaño de los mismos, ni la producción comercial.

En relación al embolsado de las granadas y la aplicación aérea de quitosanas para la profilaxis de algunas fisiopatías de la piel bajo distintas condiciones de riego, cabe mencionar que la utilización de bolsas de celulosa satinada y abiertas por el extremo inferior indujeron un cierto retraso en el crecimiento y la maduración de los frutos, aunque muy importantes beneficios pueden derivarse de su utilización ya que redujo significativamente la incidencia del albardado y aumentó la actividad antioxidante de los arilos. En cuanto a la aplicación de las quitosanas, aditivo alimentario según la USFDA, se detectó una reducción en el peso de los frutos y una disminución del color rojizo de los arilos. Sin embargo, aumentó la actividad antioxidante de los arilos y disminuyó la incidencia del albardado y el agrietado de la piel de los frutos.

Abstract

The purpose of the present study was focused in deep in the knowledge of the response of three emerging crops, persimmon (*Diospyros kaki*), pomegranate (*Punica granatum*) and quince (*Cydonia oblonga*), to water stress and to develop strategies to improve irrigation water saving and fruit quality. For this, and in order to complement the current state of knowledge in each crop, different experimental assays were performed.

As a preliminary step, the resistance mechanisms (stress avoidance and stress tolerance) developed by persimmon plants on *Diospyros lotus* in response to mild water stress and the sensitivity of continuously and discretely measured indicators of the plant water status were investigated in 3-year old ‘Rojo Brillante’ persimmon plants. The results indicated these plants confront a mild water stress situation by gradually developing stomata control (stress avoidance mechanism) and exhibiting some xeromorphic characteristic such as high leaf relative apoplastic water content, which could contribute to the retention of water at low leaf water potentials. Sap flow was seen to be the most suitable indicator for estimating persimmon water status, because it provided the highest signal intensity:noise ratio in almost all intervals of time considered and provides continuous and automated registers of the crop water status in real time.

The strategy (isohydric or anisohydric) by which quince plants cope with water stress and the resistance mechanisms developed in response to water stress and during recovery were studied in own rooted 17-years old BA-29 plants. These plants exhibited extreme anisohydric behaviour in response to water stress. Also, during the development of water stress, active osmotic adjustment take place and leaf turgor was maintained, contributing to the high leaf conductance, and, therefore, good leaf productivity. In addition, the low quince leaf apoplastic water fraction under water stress can favour water absorption because a steeper gradient in water potential between the leaf and the soil can take place. In an additional experiment, the linear fruit growth phase was seen to be a critical phenological period for the marketable yield because water deficit decreased the fruit weight, size, moisture content and fruit’s chemical characteristics. In this sense, total soluble solids increased and the total polyphenols content and antioxidant activity decreased as a result of water deficit effect. Nevertheless, the still high levels of total polyphenols, regardless of the fruit water status, suggested that quinces can be used as an important source of natural antioxidants. Also, the peel colour intensity of water stressed fruits increased and fruit flesh texture improved (corkiness and fibreness severely decreased and crunchiness increased).

In a study to determine the optimum harvest time to improve the fruit quality from Mollar de Elche own rooted pomegranate plants, fruit ripening was confirmed as a critical period because irrigation is clearly essential during most of this phenological period to achieve maximum yield. Nevertheless, a very short period of irrigation restriction (around 6 days) at the end of ripening period comes early harvest time, saves irrigation water, enhances the bioactive compounds content (anthocyanins, phenolic compounds, punicalagin and ellagic acid) without affecting marketable yield and fruit size.

In order to study if pomegranate bagging and preharvest chitosan spraying were able to affect fruit chemical characteristics and fruit peel physiopathies incidence in plants under different irrigation conditions, two experiment were performed. Fruits were bagged with externally glossy single layer cellulosic paper bags open at the bottom from the end of fruit thinning to harvest time. Despite bagging induced a delay in fruit growth and ripening, these effects were outweighed by very important commercial benefit in terms of the reduced incidence of peel sunburn and the increase in fruit antioxidant activity. Some of the effects of chitosan, a food additive by the USFDA, were negative such as the reduction in fruit weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel cracking or splitting and fruit sunburn physiopathies occurrence, which would considerably improve the returns of pomegranate growers.

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4. Introduction

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Introduction

Mediterranean agriculture is characterized by hot dry summers, mostly rainy winters and partially wet spring and autumn. For this, to ensure regular crop yields and to reduce alternate bearing pattern, the scarce rainfall has to be supplemented by irrigation in order to avoid plant water deficits (Galindo et al., 2018). In these areas, as the population increases, leading to an increasing expansion of urban, touristic and industrial activities, tension and conflict between water users and pressures on the environment will be intensified. Compounding this situation, climate change will inevitably lead to very frequent and severe droughts in the near future, so that the pressure for a more sustainable use of water in agriculture will grow because agriculture wastes 60% or 1500 trillion liters of the 2500 trillion liters of water it uses each year, which represents 70 % of the world's accessible water (Clay, 2004).

It is important to consider that in human history, 40-100,000 plant species have been regularly used not only for food but also for other purposes as fiber, industry, culture and medicine. However, modern agricultural systems have promoted the cultivation of high-input and high-yielding crop species, leading to the intense cultivation of a restricted number of species. Moreover, these species have been the focus of attention of commerce and scientific research worldwide, declining in the cultivation of many traditional fruit crops, which have resulted in a global reduction in crop diversity (Chivenge et al, 2015). However, many of these neglected or underutilized species are not only critical for the diversity of human diets, but can also contribute to increasing food production, providing a more sustainable and resilient agro- and horti-food system (Baldermann et al., 2016).

For these reasons, it is important to face up to the fact that sustainable agriculture in these agrosystems will be forced to cope with water scarcity by adopting improved, innovative and precise deficit irrigation management practices able to minimize the impact on crop yield and quality (Fernandez and Torrecillas, 2012). In addition, this type of irrigation will help to enable the diversification of fruit tree culture turning growers´ attention to a variety of underused fruit tree crops, which may need low inputs of water, synthetic fertilisers and pesticides. Besides, they yield attractive fruits, rich in nutrients and health-promoting compounds, and so have a huge potential for improving nutrition and providing preventive effects against some chronic diseases (Baldermann et al., 2016; Rodríguez et al., 2018).

Fruits grown with deficit-irrigation strategies have been aptly called *hydroSOStainables* or, in abbreviated form, *hydroSOS* (Noguera-Artiaga et al., 2016; Cano-Lamadrid et al., 2017). These *hydroSOS* products have a solid identity based on two main factors: (i) water stress can increase the plant secondary metabolite content and, thus, the functionality of the edible products (Ripoll et al., 2014), and (ii) they are environmentally friendly because of the sustainable use of a very scarce resource-water (Rodríguez et al., 2018).

It is evident that recently there is a certain tendency to change the traditional Mediterranean fruit industry, consolidating the cultivation of low water demanding and profitable Mediterranean traditional crops, such as olive, almond or grapevine and introducing new emerging crops, previously underutilized. At this last respect, quince (*Cydonia oblonga* Mill.) trees is one example of an underutilized crop species and does not require intensive maintenance and can grow in many warm-temperate and temperate regions of the world. It has also become a key factor for other crop cultures, being, for instance, the most important rootstock for pear cultivation (Gur et al., 1978). Quince fruits are also known for their hypoglycemic, anti-inflammatory, anticarcinogenic, antimicrobial, anti-allergic and antiulcerative properties and the ability to act as a tonic for heart and brain (Gur et al., 1978; Hamauzu et al., 2005; Shinomiya et al., 2009). Despite these fruits are climateric, with an attractive golden yellow colour and the above mentioned health related desirable characteristics, consumption of the fresh quince fruit or quince-based products is not widespread because they have excessive astringency, sourness and woodiness at harvest, but a pleasant, lasting, and powerful flavour when ripe (Szychowski et al., 2014). However, to date, despite the very important advantages to be had as a result of eating quince and the ever more frequent water shortages experienced in the most suitable regions for its growth, there are important gaps on the knowledge of quince plants response to drought at plant water relations level and the effects on yield, fruit physicochemical characteristics, sensory profile and volatile composition.

Persimmon (*Diospyros kaki* L. f.) is native to the mountains of central China and Japan (Mowat and George, 1994; George et al., 1997; Llácer and Badenes, 2002) and is considered underutilized or minor fruit crop species. Its culture in the Spanish Mediterranean basin is steadily increasing, aided by its high yields, high commercial value of the fruit, excellent post-harvest storage life and excellent adaptation to temperate warm climates. Nevertheless, according to Badal et al. (2010), irrigation water requirements of persimmon are quite large. So, water deficit is considered as the main environmental factor affecting unstable persimmon fruit production (Suzuki et al., 1988; Yamamura et al., 1989; Yakushiji et al., 2013). For this, irrigation may be the main limiting factor for persimmon culture in Mediterranean agrosystems due to the persistent shortage of water resources. Then, persimmon irrigation will need to be based on the use of very precise deficit irrigation management strategies that are able to significantly reduce the amount of irrigation water necessary with minimum effects on yield and fruit quality. In this sense, the use of plant-based water status indicators may be considered as an ideal tool for precise deficit irrigation scheduling in fruit trees, as has been reported by Naor (2000); Lampinen et al. (2001); García-Orellana et al. (2007); Ortuño et al. (2009a, b), Ortuño et al. (2010) and Conejero et al. (2011). When irrigation scheduling is based in the values of a plant-based water stress indicator, it has to be sufficiently sensitive, consistent and reliable for detecting minimum changes in the plant water status.

Bearing in mind that research on plant water relations of persimmon plants in response to drought is very scarce. It is key to evaluate the sensitivity of continuous and discretely measured indicators of the plant water status to use in irrigation scheduling in persimmon trees, and to analyze leaf water relations in order to clarify the response mechanisms (stress avoidance and stress tolerance) developed by persimmon plants in response to mild water stress.

Pomegranate (*Punica granatum* L.) is mainly grown in semiarid mild-temperate to subtropical climates (Blumenfeldet al., 2000) and it is considered to be a drought-resistant crop because it supports heat and thrives in arid and semiarid areas, even under desert conditions (Aseri et al., 2008). Nevertheless, for commercial production pomegranate requires regular irrigation throughout the season, especially when it is cultivated in arid and semiarid areas, to reduce the incidence of fruit physiopathies (e.g. fruit cracking or splitting) (Galindo et al., 2014b; Rodríguez et al., 2017) and to reach optimal growth, yield and fruit quality (Levin, 2006; Holland et al., 2009).

Reports on the effect of irrigation management on pomegranate fruit yield and quality are very scarce. The first results indicated that it is possible to control the desired ripening time in pomegranates by applying different irrigation regimes (Sonawane and Desai, 1989). Sulochanamma et al. (2005) showed that irrigation has a positive effect on pomegranate vegetative growth, yield, and fruit weight. Pomegranate fruit growth and ripening are considered critical phenological periods (Intrigliolo et al., 2013; Laribi et al., 2013), but it is not known if for productivity to be adversely affected it is necessary to exceed a threshold level of water stress.

In last years, the increase in pomegranate fruit consumption can be ascribed to its perceived health-related characteristics such as its antiatherosclerotic effects (Aviram et al., 2008), its high antioxidant activity (Gil et al., 2000; Seeram et al., 2006) and the anticarcinogenic compounds it contains (Malik et al., 2005; Adhami and Mukhtar, 2006; Malik and

Mukhtar, 2006). However, to fully satisfy consumers’ requirements, fruit health-related characteristics and taste have to be accompanied by the absence of pesticide residues, insect attack injuries, mechanical damage and physiopathies incidence. The incidence of some pomegranate physiopathies (mainly sunburn, cracking and splitting), is frequent, making fruits unmarketable and causing substantial economic losses to farmers, who may lose half of their crop yield (Blumenfeld et al., 2000; Melgarejo et al., 2004; Yazici and Kaynak, 2009).

Despite the important and frequent incidence of pomegranate physiopathies there have been no reports on the effect of biostimulant spray or shading on pomegranate fruit yield and the occurrence of fruit cracking or splitting and fruit sunburn physiopathies. The effect of both factors and their interaction and plant water status on fruit quality attributes has not been considered either.

Bearing in mind the aforementioned state of knowledge on the emerging crops considered and the results obtained in this doctoral thesis, it is clear that research needs to be directed at some very important aspects.

Studies on emerging crops water relations under water stress are essential because there are abundant cases in which the irrigation water requirements are not related with the resistance to water stress. The fruit water relations studies are of unquestionable importance to understand changes in fruit yield or some key fruit characteristics that are important contributors to quality, and the incidence of some fruit physiological disorders.

Considering that fruit composition response to moderate and severe water deficit is not proportional in many cases, more research is necessary to determine for each emerging crop the optimal water deficit level, its timing and duration in order to optimize fruit quality and their health-related compounds content. Based on the fact that consumers are willing to pay for special foods, particularly those associated with environmental friendly farming, it is essential to study the necessary aspects to establish a *hydroSOS* index to certify that the products using the *hydroSOS* logo have been evaluated for their sustainable use of irrigation water and/or their contents of bioactive compounds.

Even though the results on the control of pomegranate fruit physiopathies are very important, complementary research is necessary in order to optimize the use of bagging and chitosan spraying and to reduce costs.

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5. Objectives

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The overall objective of the present study was focused in **deep in the knowledge of the response of three emerging crops**, **persimmon** (*D. kaki*), **pomegranate** (*P. granatum*) **and quince** (*C. oblonga*), **to water stress and to develop strategies to improve irrigation water saving and fruit quality**

To this end the following partial objectives were addressed:

- *To evaluate the sensitivity of continuous and discretely measured indicators of the plant water status to use in irrigation scheduling in persimmon trees and to clarify the stress avoidance and stress tolerance mechanisms developed in response to mild water stress.*

- *To increase knowledge on the strategy (isohydric or anisohydric) by which quince plants cope with water stress and to elucidate the resistance mechanisms involved (avoidance and tolerance).*

- *To look at the effect of water stress on quince yield, the main physicochemical characteristics, sensory profile and volatile composition.*

- *To evaluate whether water restrictions during the pomegranate fruit ripening stage have secondary effects on yield, fruit characteristics and composition.*

- *To analyse the interaction between preharvest pomegranate fruit chitosan spraying and plant water status on yield, fruit quality attributes, and the occurrence of fruit cracking or splitting and fruit sunburn physiopathies.*

- *To evaluate the effect of bagging on pomegranate fruit attributes and fruit peel physiopathies incidence under full irrigation and water stress conditions.*

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6. Materials and Methods

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In this section the main characteristics of the experimental conditions, irrigation treatments, analytical methodologies, statistical design and analysis, etc. used in this thesis are included. For more detailed aspects the papers that constitute this thesis can be consulted.

Plant Material, experimental conditions and treatments

The Harisa farm

The farm is farm located near the city of Murcia (Spain) (38°1'N, -1°3'W). The soil is a Calcaric fluvisol with clay texture. Soil volumetric water contents (θv) at saturation, field capacity and permanent wilting point were 0.48, 0.42 and 0.28 $m^3 m^{-3}$, respectively. The irrigation water had an electrical conductivity of between 1.2 and 1.4 dS/m and a Cl^- concentration ranging from 20 to 35 mg L^{-1}.

The climate of the area is typically Mediterranean, with mild winters, low annual rainfall, and hot dry summers. During the experimental period, average daily maximum and minimum air temperatures were 32 and 19 ºC, respectively, the mean daily air vapour pressure deficit (VPD_m) (Allen et al., 1998) ranged from 0.89 to 2.64 kPa, and reference crop evapotranspiration (ETo), (Allen et al., 1998) was 171 mm. No rainfall was recorded during the experimental period.

The plant material consisted of 3-year old persimmon trees (*Diospyros kaki* L. f. cv. 'Rojo Brillante' grafted on *Diospyros lotus* L.). Tree spacing followed a 3 m x 5 m pattern. Pest control and fertilization practices were those normally used by the growers (Photograph 7.1).

Two irrigation treatments were considered, in which irrigation was carried out daily and during night time using a drip irrigation system with one lateral pipe per tree row. From day of the year (DOY) 218 to 251, in order to guarantee non-limiting soil water conditions, control plants (treatment T0) were irrigated using six emitters (each delivering 4 l h^{-1}) per plant in order to maintain soil water content in the 0 - 60 cm soil depth at levels near constant and slightly above soil field capacity. In the T1 treatment water was applied at 70 % of control trees.

Photograph 7.1. Persimmon (*Diospyros kaki* L. f. cv. Rojo Brillante) trees at the *Harisa* farm.

The EPSO experimental farm

The experimental plot was located at the EPSO farm of the Miguel Hernández Polytechnic University of Elche, near the city of Orihuela (Spain) (38º 4'N, 0º 59'W). The soil is a Xerofluvent, with a high active calcium carbonate and low organic matter content; available potassium and phosphorus levels, and cationic exchange capacity were also low.

The plant material consisted of own rooted adult quince trees (*Cydonia oblonga* Mill.), cv. BA-29, planted at 4 m × 5 m. The orchard was managed under no tillage conditions; weeds were controlled with post-emergence herbicides. Pest control and fertilization practices were those usually used by local growers (Photograph 7.2).

Irrigation was carried out daily and during the night using a drip irrigation system with one lateral pipe per tree row. Irrigation water had a concentration of 71 - 84 mg L^{-1} and an electrical conductivity of between 1.4 and 1.6 dS m^{-1}. Two irrigation treatments were applied during summer. Control plants (T0) were irrigated using six emitters (each delivering 3 l h^{-1}) to ensure non-limiting soil water conditions (105 % ETo). T1 plants were irrigated as T0, except that irrigation was withheld during the linear fruit growth phase (DOY 209-251), before restoring irrigation at the same level as used in T0 from DOY 251 to 266 (recovery period). During the experimental period, T0 and T1 received a total of 374 and 143 mm water, respectively, including rain water.

Photograph 7.2. Quince (*Cydonia oblonga* Mill. cv BA-29) trees at the *EPSO* experimental plot.

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The Hacienda San Miguel farm

The Hacienda San Miguel farm is located near the city of Alhama de Murcia (Spain). The pomegranate (*Punica granatum* L.) trees (37°47′N, 1° 25′W) were own-rooted 15 years old Mollar de Elche cultivar and the tree spacing was 3 m × 5 m. The soil of the orchard is a moderately saline silt loam (Hyposalic Calciorthid), with moderate lime content, very low organic matter content, low cationic exchange capacity, high available phosphorus levels and low available potassium (Photograph 7.3).

Pest control and fertilization practices were those usually used by local growers, and no weeds were allowed to develop within the orchard. The irrigation water used had an electrical conductivity of between 0.9 and 1.3 dS m^{-1}. The chloride (Cl^{-}) concentration in the irrigation water ranged from 67 to 78 mg L^{-1} during the experimental period.

During the growing season, control plants (T0) were irrigated above crop water requirements (123 % ETc) in order to ensure non-limiting soil water conditions. Irrigation was performed daily during the night using a drip-irrigation system with a lateral pipe parallel to each tree row and 3 emitters per tree, each delivering 4 L h^{-1}. In-line water meters were used to measure the water supplied to each experimental unit. T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277-283, fruit late ripening), 15 (DOY 268-283, second half fruit ripening), 25 (DOY 258-283, fruit ripening) and 36 (DOY 247-283, end fruit growth and late ripening) days before harvest (DOY 283), respectively, when irrigation was withheld. The total amount of water received by each treatment during the experimental period (DOY 247-283) was 128, 110, 86, 49 and 0 mm for T0, T1, T2, T3 and T4 treatments, respectively, without considering precipitation (basically the 84 mm that fell on DOY 271).

Photograph 7.3. Pomegranate (*Punica granatum* L. cv Mollar de Elche) tree at the *Hacienda San Miguel* experimental plot.

The Finca Tres Caminos Experimental Station

The plant material consisted of own-rooted 7-year old pomegranate plants (*P. granatum* (L.) cvs. Mollar de Elche and Wonderful) spaced following a 3 m × 5 m pattern (38° 6′ N; 1° 2′ W), which is located in the *Tres Caminos* Experimental Station near Santomera (Murcia, Spain). The soil of the plot is stony (33%, w/w) and shallow, with a clay-loam. The orchard was managed under *no tillage* conditions; weeds were controlled with post-emergence herbicides. Pest control and fertilization practices were those usually used by local growers (Photograph 7.4).

Photograph 7.4. Pomegranate (*P. granatum* L.) cvs. Mollar de Elche (left) and Wonderful (right) trees at the *Finca Tres Caminos* experimental plot.

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Irrigation was performed daily during the night using a drip-irrigation system with a lateral pipe parallel to each tree row and four emitters per tree, each delivering 4 L h^{-1}. In addition, in-line water meters were used to measure the water supplied to each experimental unit.

- Bagging assay

Fully irrigated plants (treatment FI) were irrigated during the night to above crop water requirements - 115% evapotranspiration (ETo) - using a drip irrigation system with one lateral pipe per tree row and four emitters (each delivering 4 L h^{-1}) per plant. Irrigation in water-stressed plants (treatment WS) was withheld for 60 days (from day of the year, DOY, 209 to 269), when evaporative demand is very high and water availability for irrigation is very scarce. To guarantee the recovery of WS plants, re-irrigation was performed at the levels used in FI from DOY 269 to 286 (harvest time). From DOY 209 (the end of fruit thinning) to harvest, pomegranate fruits from both irrigation treatments were submitted to bagging with Pantone® 1205C colored bags (262 mm × 397 mm) open at the bottom, made from externally glossy single-layer cellulosic paper (grade: 50 gm^{-2}, Bendtsen porosity: 373 mL min^{-1}) and stapled tightly around the fruit peduncle (Photograph 7.5). All fruits from the treatment trees were bagged, and formed treatment B, while treatment NB consisted of fruits that were not bagged. For prophylactic purposes, plants were sprayed with fungicide (containing 80% sulfur) and insecticide (10% of 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether) a week before the bagging practice.

Photograph 7.5. Mollar de Elche pomegranate bagged fruits at the *Finca Tres Caminos* experimental plot.

- Chitosan assay

Fully irrigated plants (FI) were irrigated above the estimated crop water requirements (115 % crop reference evapotranspiration, ETo) while irrigation was withheld from the water stressed plants (WS) from the day of the year (DOY) 221 to DOY 269 (48 days), after which, irrigation was resumed at FI level until harvest (DOY 286, 13 October). In addition, on day of the year, DOY, 221 (10 days after the end of fruit thinning) and on DOY 254, plants from both irrigation treatments were sprayed with a Quitomax® solution at 45 g of active ingredient per ha. This active ingredient consists of chitosan polymers of medium molecular weight (≥ 100 kDa), obtained with basic deacetylation from chitin. Plants treated with Quitomax® comprised treatment Q, while treatment NQ consisted of plants that were not sprayed with chitosan.

Measurements

Weather and plant water relations

Using automatic weather stations, placed near the experimental plots, the following parameters were measured: wind speed 2 m above the soil surface, rainfall, solar radiation, air temperature and air relative humidity. Daily values of crop reference ETo were calculated using the Penman-Monteith equation (Allen et al., 1998). Mean daily air vapour pressure deficit (VPDm) was calculated according to Allen et al. (1998).

θv was measured with a portable FDR sensor (HH2, ΔT, U.K.) previously calibrated. The access tubes for the FDR sensor were placed in the irrigation line at about 30 cm from an emitter. The data were obtained at 0.10, 0.20, 0.30, 0.40 and 0.60 m depth.

Predawn leaf water potential (Ψ_{pd}), Fruit water potential (Ψ_{fruit}), leaf water potential (Ψ_{leaf}), and midday stem water potential (Ψ_{stem}) were measured in two fruits or two leaves located on the south facing side, from the middle third of the tree, using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), as recommended by Turner (1988), McFadyen et al. (1996) and Galindo et al. (2014b). Leaves for Ψ_{stem} measurements were enclosed in a small black plastic bag covered with aluminium foil for at least 2 h before the measurements were made. Leaf conductance (g_{leaf}) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree similar to those used for Ψ_{pd} measurements.

In order to assess the cumulative effect of the water deficit, the water stress integral (SI) was calculated from the g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data according to the expression proposed by Myers (1988).

$$SI_A = \left|\sum (\bar{A} - H)\, n\right|$$

where A can be g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} and $\bar{A}$ is the average g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} value for any interval, H is the maximum value measured during each interval and n is the number of days in the interval.

After measuring leaf water potential at any time, the leaves were frozen in liquid nitrogen and the osmotic potentials were measured after thawing the samples and expressing sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Leaf turgor potentials were derived as the difference between osmotic and water potentials.

Estimates of predawn leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (€), relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) were obtained from pressure-volume (PV) analyses of leaves (Tyree and Hammel, 1972; Tyree and Richter, 1981, 1982). At the end of the stress period, leaves were sealed in plastic bags immediately after excision and resaturated by dipping their petioles in distilled water for 24 h at 4 ºC. The resaturated leaves were weighed using an analytical balance (± 0.1 mg precision), placed in the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber). After being depressurized, the leaf was allowed to transpire outside the pressure chamber on the laboratory bench at room temperature (22 ± 2 ºC). Leaves were repeatedly weighed and their balance pressures determined over the full range of the pressure gauge (Kikuta and Richter, 1986). Data for initial saturated weight, intermediate fresh weight (corresponding to values for Ψ_{leaf}), and final dry weight (at 80 ºC for 48 h) were used to calculate the relative water content (RWC) (Barrs and Weatherley, 1962).

The curves were drawn using a type II transformation (Tyree and Richter, 1982). When the reciprocal of water potential (Ψ_{leaf}) was plotted against RWC, the resultant relationships displayed both linear and non-linear regions. Extrapolation on the straight portion of the curve obtained for a value of RWC = 1 gave the reciprocal of the Ψ_{os} and extrapolation to the abscissa gave RWC_a. Ψ_{tlp} and RWC_{tlp} were estimated as the intersection between the linear and curvilinear portions of the PV curve. The bulk modulus of elasticity (Є) of leaf tissue at 100% RWC (RWC_o) was estimated according to Patakas and Noitsakis (1999) as Є (MPa) = $(\Psi_{os} - \Psi_{stlp})(100 - RWC_a)/(100 - RWC_{tlp})$, where Ψ_{stlp} is the osmotic potential at the turgor loss point and Ψ_{os} values correspond to those obtained from the analysis of the PV curves.

The methodology proposed by Martínez-Vilalta et al. (2014) was used to categorize the strategy (isohydric or anisohydric) by means of which plants cope with drought stress. The above authors assumed that, within biologically reasonable ranges of water potentials, the relationship between soil water potential (Ψ_s) and minimum leaf water potential (Ψ_L) becomes linear ($\Psi_L \approx \Lambda + \sigma\ \Psi_s$), assuming that soil and plant water potential equilibrate overnight so that Ψ_{pd} and Ψ_{md} are proxies of Ψ_s and Ψ_L, respectively. The slope (σ) indicates the rate of reduction in Ψ_L as Ψ_s declines. Specifically, (i) when Ψ_L remains constant as Ψ_s diminishes ($\sigma = 0$), plants show a strictly isohydric behaviour, (ii) when the difference between Ψ_L and Ψ_s remains constant ($\sigma = 1$), plants show a strictly anisohydric behaviour, (iii) when the difference between Ψ_L and Ψ_s is reduced as Ψ_s diminishes ($0 < \sigma < 1$), plants show a partial isohydric behaviour, and (iv) when the pressure drop increases through the plant as Ψ_s diminishes ($\sigma > 1$), plants show an extremely anisohydric behaviour.

The micrometric trunk diameter fluctuations (TDF) were measured throughout the experimental period on four trees per treatment, using a set of linear variable displacement transducers (LVDT) (model DF ±2.5 mm, accuracy ±10 μm, Solartron Metrology, Bognor Regis, UK) attached to the trunk, with a special bracket made of Invar, an alloy of Ni and Fe with a thermal expansion coefficient close to zero (Katerji et al., 1994), and aluminium. Sensors were placed on the north side, 10 cm above the graft point of each tree, and were covered with silver thermoprotected foil to prevent heating and wetting of the device. Measurements were taken every 10 s and the datalogger (model CR10X, Campbell Scientific, Logan, UT, USA) was programmed to report 15 min means. MDS was calculated as the difference between maximum and minimum daily trunk diameter.

SF was measured using the compensation heat-pulse technique (Swanson and Whitfield, 1981) in the same trees used for TDF measurements throughout the experimental period. One set of heat pulse probes was located above the LVDT sensors on each tree. Each set consisted of a heater needle of 1.8 mm diameter and two temperature probes also of 1.8 mm diameter installed in parallel holes drilled radially in the trunks at 10 mm downstream and 5 mm upstream. Each heat-pulse probe had three thermocouple sensors to monitor the sap velocity at a radial depth of 5, 12 and 21 mm below the cambium. Sap velocity was measured following the procedure of Green et al. (2003), using the theoretical calibrations of Swanson and Whitfield (1981) to account for the probe-induced effects of wounding. The volume fractions of wood and water determined by López-Bernal et al. (2014) were used. The temperature signals and the corresponding heat-pulse velocities were recorded at 30 min intervals using heat-pulse instrumentation controlled by a datalogger (CR10X, Campbell Scientific Ltd., Logan, Utah)

Yield, morphology, fruit phisiophaty incidence and colour

At harvest, the incidence of each fruit disorder was determined by counting the number of healthy or specific disorder-affected fruit. The mean fruit weight of the marketable fruit yield was determined according to the weight and number of healthy fruits per box in two randomly selected boxes per replicate

Measurements of the polar and equatorial diameters and peel thickness of the fruit were made using a digital calliper. Flesh firmness was determined as the load needed to break the flesh after removal of the peel on two opposite faces in the equatorial zone, using a penetrometer (model FT-327, Facchini, Alfonsine, Italy), equipped with an 8 mm cylindrical plunger.

For peel colour, 4 different measurements at 4 equidistant points on the equatorial region of each fruit were performed and for arils colour the arils in each fruit were spread on a white plate and their colour was assessed in ten different places of the plate. In both cases the measurements were assessed using a Minolta CR 2000 colourimeter (Osaka, Japan) and the results were expressed in the CIE$L^*a^*b^*$ system. The mean values for lightness (L^*), red-greenness (a^*), and blue-yellowness (b^*) coordinates for each fruit were reported. In addition, the objective colour was calculated as chromaticity or chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$H^o = \arctan(b^*/a^*)$].

Fruit total soluble solids, acidity, maturity index and moisture content

Total soluble solids and total acidity were measured in juice samples according to Rodriguez-Guisado (2009), and the maturity index was calculated as the ratio between both parameters. For the moisture content, samples were oven-dried at 105 ºC until constant weight (AOAC, 2000).

Organic acids and sugars

Organic acids and sugars (citric acid (CA), succinic acid (SA), glucose (Glu) and fructose (Fru)) were quantified according to Melgarejo-Sánchez et al. (2015). For this, 20 mL of juice was centrifuged at 15,000 × g for 20 min (Sigma 3–18K, Osterode & Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm cellulose nitrate membrane filter and the samples (10 µL) were injected onto a heated (30 ºC) Supelcogel TM C-610H column (30 cm × 7.8 mm i.d., Supelco, Bellefonte, PA, USA) protected with a Supelcogel C610H guard column (5 cm × 4.6 mm, Supelco, Inc.). The HPLC system used was a Hewlett-Packard 1100 series model (Wilmington Del., USA) with autosampler and UV detector, set at 210 nm, coupled to a refractive index detector (HP 1100, G1362A). The elution system consisted of 0.1% phosphoric acid at a flow rate of 0.5 mL/min. Standard curves of pure organic acids and sugars were used for the quantification. Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

Total anthocyanin content, total phenolics content and total antioxidant activity of the fruit

The total anthocyanin content was determined by a pH differential method with two buffer systems, sodium acetate buffer, pH 4.5 (0.4 mol L^{-1}) and potassium chloride buffer, pH 1.0 (0.025 mol L^{-1}) (Giusti et al., 1999). Fruit juice (0.4 mL) was mixed with 3.6 mL of the corresponding buffers and read against water as blank at 510 nm and 700 nm. The absorbance (A) was calculated as $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$. The total anthocyanin content was calculated by following the equation TAC = (A × MW × DF × 100/MA), where A is the absorbance, MW is the molecular weight (449.2), DF is the dilution factor (10), and MA is the molar absorptivity of cyaniding-3-glucoside (26.900). The result was expressed as mg cyaniding-3-glucoside (C3G) L^{-1} of fruit juice.

The total phenol content (TPC, mg GAE 100 g^{-1}) of fruits was estimated using the Foling-Ciocalteu reagent following the recommendations of Singleton et al. (1999).

The antioxidant activity was evaluated with three different freeze methodologies ($ABTS^{+}$, FRAP and $DPPH^{\bullet}$). The $ABTS^{+}$ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation and ferric-reducing antioxidant power (FRAP) methods were applied according to Re et al. (1999), and Benzie and Strain (1996), respectively. The radical scavenging activity was evaluated using the DPPH$^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995) with a modification in the reaction time (Nuncio-Jáuregui et al., 2015).

Punicalagin isomers and ellagic acid

The punicalagin (isomers α and β) and ellagic acid contents were determined in freeze-dried fruits (0.3 g) diluted with 7 mL of MeOH/water (80:20, v/v) and 1% acetic acid and then centrifuged at 15,000 rpm for 20 min. Supernatants were filtered through a 0.45 µm Millipore filter and then injected into a Hewlett-Packard series 1200 HPLC equipped with a diode-array detector. Each sample (20 mL) was analysed on a LiChroCART 100 RP-18 reversed-phased column (250 mm x 4 mm, particle size, 5 µm; Merck, Darmstadt, Germany) equipped with a C18 pre-column (LiChrospher 100 RP-18, 5 mm; Merck, Darmstadt, Germany) using a mobile phase of 1% acetic acid in ultra-high purity deionised water (solvent A) and 1% acetic acid in MeOH (solvent B). Elution was performed at a flow rate of 1 mL min^{-1} using a gradient starting with 1% B for 5 min, and increasing to 60% B for 40 min. Punicalagin (α and β) and ellagic acid were detected at 360 nm. To confirm their identification, absorption spectra and retention times were compared with those obtained from chemical standards. Standard curves for pure punicalagins (Chengdu Biopurify Phytochemicals Ltd., Sichuan, China), with a concentration range of 0.05 - 0.80 g L^{-1}, as well as for ellagic acid (Tocris Bioscience, Ellisville, MO), with a concentration range of 0.0025 - 0.0200 g L^{-1}, were used for quantification. The results for individual isomer punicalagin (α and β) and ellagic acid are expressed as mg L^{-1}.

Fruit sensory analysis

Trained panellists from the Food Quality and Safety Group of the Universidad Miguel Hernández de Elche, UMH (Alicante, Spain) participated in the studies. Each of the panellists had more than 750 h of testing experience with a variety of food products. For each study, the panellists received further orientation on fresh and processed fruits.

The evaluation was carried out at the UMH facilities using individual booths with a combination of natural and non-natural (fluorescent) light (70–90 fc) and a temperature of 20 ± 2 ºC during three different sessions. Samples were evaluated in triplicate and the sampling order for each panellist was randomized.

The fruit samples were served in odour-free, disposable 100 mL covered plastic cups. Three slices of quince fruit (approximately 40-50 g; each slice from a different fruit) were served to each panellist; additional samples were available if the panellists requested it. All samples were served at room temperature. Unsalted crackers and distilled water were used to clean the palate between samples.

Three sessions were held for the sample evaluation, and fruits from each treatment were assayed in each session. Fruit samples were assessed using descriptive sensory analysis. Each panellist had a questionnaire to evaluate the following attributes: colour (peel colour and colour homogeneity), odour (quince, fruity, pineapple, apple and pear), flavour (sourness, sweetness, bitterness, and astringency), aroma (fruity, quince, pineapple, pear, and aftertaste) and texture (hardness, crunchiness, juiciness, graininess and fibreness). The panel developed its own sensory lexicon to describe the quality of fruits. The attributes and definitions used for the present study are shown in the results section. A numerical scale, from 0 to 10 with increments of 0.5, was used, where 0 represents no intensity and 10 represents extremely strong intensity. Results are reported as the mean value ± standard error.

Volatile aroma compounds analysis

Volatile compounds were extracted from fruit samples using headspace solid phase micro-extraction (HS-SPME). After several preliminary tests to optimize the extraction system, 5 g of milled fruit plus 10 mL of distilled water were placed into a 50 mL vial hermetically sealed with a polypropylene cap and a polytetrafluoroethylene (PTFE)/silicone septum. One microliter of the internal standard (nonanal) was added together with NaCl and the vial was equilibrated for 15 min at 40 °C in a water bath. Then a 50/30 μm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibre was exposed to the sample headspace for 50 min at 40 °C.

Isolation and identification of the volatile compounds were performed using a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu QP-5050A mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The GC/MS system was equipped with a TRACSIL Meta.X5 column (95 % dimethylpolysiloxane/5 % diphenylpolysiloxane, 60 m × 0.25 mm, 0.25 μm film thickness; Teknokroma S. Coop. C.Ltd, Barcelona, Spain).

The quantification (mg kg^{-1} dry weight, dw) of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC-MS analysis. The injector temperature was 200 ºC and nitrogen was used as carrier gas (1 mL min^{-1}). The relative abundance was obtained from electronic integration measurements using flame ionization detection (FID). Nonanal was used as internal standard and the areas from all compounds were normalized using its area; this compound was chosen after checking that it was not present in the volatile profiles of the samples under study.

Statistical design and analysis

The experiments had completely randomized design, with four replications, each replication consisting of three adjacent tree rows. Measurements were taken on the inner plants of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), while the other plants served as border plants.

Data were analyzed using SPSS software (SPSS, 2002). Analysis of variance was performed and mean values were compared by Tukey$_{0.05}$

test. Values for each replicate were averaged before calculating the mean and the standard error of each treatment. Percentage values were arc-sin-transformed before statistical analysis when they were not normally distributed.

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7. Publications

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7.1 Publication 1 (Open Access)

Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems

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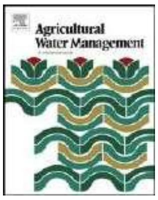

Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems

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ABSTRACT

Water scarcity in Mediterranean climate areas will be progressively aggravated by climate change, population increase and urban, tourism and industrial activities. To protect water resources and their integrity for future use and to improve biodiversity, besides following advanced deficit irrigation strategies in fruit cultivation, attention could well be directed towards what are at present underused plant materials able to withstand deficit irrigation with minimum impact on yield and fruit quality. To this end, the state of the art as regards deficit irrigation strategies and the response of some very interesting emerging fruit crops [jujube (*Zizyphus jujuba* Mill.), loquat (*Eriobotrya japonica* Lindl.), pistachio (*Pistacia vera* L.) and pomegranate (*Punica granatum* L.)] are reviewed. The strengths and weaknesses of deficit irrigation strategies and the mechanisms developed by these emerging fruit crops in the face of water stress are discussed. The response of these crops to deficit irrigation, with special attention paid to the effect on yield but also on fruit quality and health-related chemical compounds, was analysed in order to assess their suitability for saving water in Mediterranean semiarid agrosystems and to analyze their potential role as alternatives to currently cultivated fruit crops with higher water requirements. Finally, the fac- tors involved in establishing an identity brand (*hydroSOS*) to protect fruits obtained under specific DI conditions are discussed.

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1. Introduction

Mediterranean climate countries include not only those that border the Mediterranean Sea (from Spain to Turkey and Cyprus and from Morocco to Syria) but also other regions of the planet, including Southern California, Chile, South Africa and Southern Australia. All are characterized by hot dry summers, mostly rainy winters and partially wet spring and autumn. In these region, to ensure regular crop yields and for to reduce inter-annual yield variability, the scarce rainfall has to be supplemented by irrigation in order to avoid plant water deficits. Indeed, water scarcity in these sites is destined to gradually become worse because more frequent and severe droughts event driven by climate change (Collins et al., 2009). Moreover, as the population increases, leading to an increasing expansion of urban, touristic and industrial activities, tension and conflict between water users and pressures on the environment will be intensified.

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Consequently, and considering that Mediterranean agrosystems are very important consumers of fresh water, it is of paramount importance to protect water resources and their integrity for future use (Katerji et al., 2008). In this sense, to overcome the problems associated to a boost in water prices, as the discouragement of farm- ers and ultimately land abandonment, García-Tejero et al. (2014) indicated that an alternative could be to provide correct incentives for farmers to adopt changes in their irrigation methods by implementing strategies and tools for sustainable water saving. Among the strategies that can be applied to attain water saving are the use of improved, innovative and precise deficit irrigation (DI) management practices able to minimize the impact on crop yield and quality (Fernandez and Torrecillas, 2012). In addition, in order to contribute to water saving, fruit culture should be directed towards the use of plant materials that are less water-demanding or able to withstand deficit irrigation with minimum impact on yield and fruit quality

In this last respect, it is important to consider that in human history, 40–100,000 plant species have been regularly used for food, fiber and for industrial, cultural and medicinal purposes. Today, at least 7000 cultivated species are in use around the world. However, in recent centuries, agricultural systems have promoted the cultivation of a very limited number of crop species. While these have been the focus of attention of commerce and scientific research world-wide, many crops have been relegated to the status of neglected or underutilized crop species, and largely ignored (Padulosi et al., 2001; Chivenge et al., 2015). In addition, this reduction in the number of crop species used for food production throughout the world has a direct effect on biodiversity, which is fundamental for ecosystem functioning, sustainable agricultural production and the attainment of food and nutritional security (Toledo and Burlingame, 2006; Chappell and LaValle, 2011). There- fore, to improve not only biodiversity but also to saving water and hence protecting the integrity of water resources for the future, it is necessary the diversification of production and consumption habits, including the use of a broader range of plant species, in particular those currently identified as underutilized and needing a low input of synthetic fertilisers, pesticides and water. This option has to be compatible with the consolidation of the cultivation of other Mediterranean traditional crops, such as olive, almond or grapevine, which are low water demanding and profitable crops. In this sense, in some countries, during recent decades there has been a certain interest in diversifying fruit tree production by cultivating species with under-exploited potential. Among these emerging crops many are characterized by their attractive fruits and health- related qualities, so that they may attract consumer attention and contribute to producer profitability

For these reasons, the aim of this review was to present the state of the art of deficit irrigation strategies and the response to them of some very interesting emerging fruit crops [jujube (*Zizyphus jujuba* Mill.), loquat (*Eriobotrya japonica* Lindl.), pistachio (*Pistacia vera* L.) and pomegranate (*Punica granatum* L.)]. To this end, the following aspects were considered: (i) the strengths and weaknesses of deficit irrigation strategies, (ii) the mechanisms developed by these emerging fruit crops to confront water stress, and (iii) the response of these crops to deficit irrigation, paying special attention not only to the effect on yield but also to the effect on fruit quality and health-related chemical compounds.

2. Deficit irrigation. Concepts and strategies

To cope with water scarcity, Mediterranean agrosystems are increasingly looking to more efficient technological innovation and irrigation management approaches. In this respect, many countries have shifted from irrigating crops in order to satisfy their evapotranspiration requirements (ETc) or full irrigation (FI), the conventional norm which seeks to maximize crop yield per unit of land, to deficit irrigation (DI) strategies, which involve reducing the amount of water provided to the crop during the growing season by the soil moisture stock, rainfall and irrigation to a level below that needed for maximum plant growth. In most of cases DI induces a gradual water deficit, due depletion of soil water reserves, accompanied by a reduction in harvestable yields, especially in soils with a significantly low water storage capacity.

When water scarcity is the consequence of uncontrolled factors and water supply is not guaranteed, farmers find it difficult to schedule any reasonable DI strategy. In contrast, if growers have a guaranteed water supply for their crops during the growing season, it is possible to improve water productivity (WP) by drawing up DI strategies based on scientific principles, attempting to produce near-maximum yields even if crops are provided with less water than they would otherwise use (maintaining crop consumptive use below its potential rate). In other words, improving the marketable yield per unit of water used rather than attaining maximum yields (Kijne et al., 2003; Zhang, 2003) Complementary advantages of the same include a reduction of nutrient loss from the root zone and a decrease in excessive vegetative vigour, accompanied by a lower risk of crop diseases linked to high humidity (Goodwin and Boland, 2002; Ünlü et al., 2006) (Table 1). However, there is a shortage of research into the risk of soil salinization as a consequence of any decrease in the leaching of salts and the use of low quality irrigation water (Boland et al., 1996; Kaman et al., 2006) (Table 2).

Three main DI strategies can be mentioned; sustained deficit irrigation (SDI), in which irrigation water used at any moment during the season is below the crop evapotranspiration (ETc) demand, and two others, both based on physiological aspects of the response of plants to water deficit regulated deficit irrigation (RDI) and partial root-zone drying (PRD) (Fig. 1).

2.1. Sustained deficit irrigation (SDI)

At the end of 1970s, trials applying irrigation water amounts below the ETc demand but at very frequent intervals took place with encouraging results. Called deficit high-frequency irrigation (DHFI), this strategy proved unsuccessful when little water was stored in the soil. It was only possible to use DHFI and obtain maximum yields when ETc was reached through the combination of irrigation water applied and soil water depletion (Fereres et al., 1978).

In fact, the DHFI strategy is very similar to SDI (Fig. 1), which is based on the idea of allotting the water deficit uniformly over the whole fruit season, thus avoiding the occurrence of serious plant water deficit at any crop stage that might affect marketable yield or fruit quality, or distributing the irrigation water proportionally to irrigation requirements throughout the season.

2.2. Regulated deficit irrigation (RDI)

RDI works on the premise that transpiration is more sensitive to water deficit than photosynthesis and fruit growth, and water deficit-induced root-sourced chemical signals like ABA. Thus, fruit trees cope with a reduced water supply by reducing transpiration (stomata regulation or reducing leaf surface area through reducing leaf growth) (Wilkinson and Hartung, 2009). In this sense, fruit tree sensitivity to water deficit is not constant during the whole growing season, and a water deficit during particular periods may benefit WP by increasing irrigation water savings, minimizing or eliminating negative impacts on yield and crop revenue and even improving harvest quality (Chalmers et al., 1981; McCarthy et al., 2002; Domingo et al., 1996) (Table 1). Therefore, when a RDI strategy is applied, full irrigation is supplied during the drought- sensitive phenological stages (critical periods) of fruit trees and irrigation is limited or even unnecessary if rainfall provides a minimum supply of water during the drought-tolerant phenological stages (non-critical periods) (Chalmers et al., 1981;

Table 1
Key advantages of deficit irrigation (DI) strategies: sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and partial root drying (PRD) with a non-exhaustive list of references.

DI strategy	Advantage	References
SDI, RDI and PRD	Maximize the water use efficiency and water productivity (WP)	Liu et al. (2006a); Liu et al. (2006b); Saeed et al. (2008); Geerts and Raes (2009); Ahmadi et al. (2010).
	Minimum impacts on yields can be achieved when precision tools are used to manage mild DI	García-Orellana et al. (2007); Ortuño et al. (2009)
	Reduces nutrient loss from the root zone, improving ground water quality and lowering fertilizer needs on the field.	Ünlü et al. (2006) ; Goodwin and Boland (2002)
	Decrease the risk of crop diseases linked to high humidity	Goodwin and Boland (2002);
RDI	Improves water savings and even harvest quality	Chalmers et al. (1981); McCarthy et al. (2002)
	Reduces excessive vegetative vigour	Goodwin and Boland (2002).
	Can be scheduled using only trunk diameter sensors	Conejero et al. (2011); Girón et al. (2015).
PRD	It can be operated in furrow or drip-irrigated crops	Grimes et al. (1968); Samadi and Sepaskhah (1984).
	Despite a reduction in stomatal conductance, crops maintain a favourable water status	Santos et al. (2003); Kang and Zhang (2004)
	The quantity and quality of the harvest can be improved as a consequence of carbohydrates partitioning between the different plant organs	Kang and Zhang (2004)

Table 2
Key constraints of deficit irrigation (DI) strategies: sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and partial root drying (PRD) with a non-exhaustive list of references.

DI strategy	Constraint	References
SDI, RDI and PRD	At all times it is essential to access to a minimum quantity of water, below which DI has no significant beneficial effect	Zhang (2003)
	Shortage of research on soil salinization risks as a consequence of the decrease of leaching of salts and the use of low quality irrigation water.	
	Crops sustain some degree of water deficit and some yield reduction except when soil water depletion supplements irrigation to reaching ETc	Fereres et al. (1978); Costa et al. (2007)
SDI	Yield decrease is due mainly to decrease in fruit weight	Castel and Buj (1990)
RDI	The maintenance of plant water status within narrow limits of water deficit during non-critical phenological periods. Sudden change in evaporative demand risks severe losses of yield and fruit quality	Jones (2004)
	New and more precise criteria for defining water deficit are needed, because criteria based on ETc can have unpredictable final effect on the rhythm of water deficit development across a range of different growing conditions (species, weather, soil depth, fruit load, rootstock).	Shackel et al. (1997); Marsal et al. (2008)
	Irrigation management in heavy and deep soils because soil water depletion and refill can take place too slowly	Girona et al. (1993)
	Scarcity of detailed studies to know the effect of water deficit on bud development	Naor et al. (2005); Marsal et al. (2008)
PRD	Do not exist definite solid criteria on defining the optimum timing of irrigation for each root system side	Saeed et al. (2008)
	It is not possible to have absolute control of root drying under field conditions and hydraulic redistribution from deeper to shallower roots may prevent the clear results that can be obtained in potted plants	Bravdo (2005)

Mitchell and Chalmers, 1982; Geerts and Raes, 2009) (Fig. 1).

Stone fruit growth follows a double-sigmoidal pattern with two periods of rapid growth separated by a period during which little or no expansive growth occurs. The first growth period, stage I, is due to cell division and cell expansion; stage II is the period in which sclerification of the fruit endocarp takes place and fruit growth is extremely slow or null, and stage III is the second period of fruit growth, which is rapid due to the expansion of existing cells and extends from the onset of this second growth period until maturity. Pome and *Citrus* fruits show only a phase of rapid fruit growth (single-sigmoidal pattern), which takes place after the initial period of cell division and minimal expansion, and is due mainly to a cell expansion process even though some cell division may also take place at the beginning (Rodríguez et al., 2017).

In stone fruit trees, two critical periods have been identified. The first one corresponds to the second rapid fruit growth period (stage III), when drought stress induces a reduction in yield due to the smaller fruit size at harvest, and the second critical period is the early postharvest period, when drought stress affects flower bud induction and/or the floral differentiation processes that occur at this time. This leads to a lower germination potential in the pollen of the next bloom and encourages young fruit to drop in the following season (Uriu, 1964; Ruiz-Sánchez et al., 1999; Torrecillas et al., 2000). In other *Prunus* species, such as almond (*Prunus dulcis* (Mill.) D.A. Webb), flowering and rapid vegetative and fruit growth stages (stages II and III) and postharvest (stage V) have been reported as critical periods because water deficit affects yield (Goldhamer and Smith, 1995; Goldhamer and Viveros, 2000; García-Tejero et al., 2017).

In pome and *Citrus* fruits rapid fruit growth can be considered as a common critical period. In an experiment in Fino lemon (*Citrus limon* (L.) Burm. fil.) trees over four seasons,

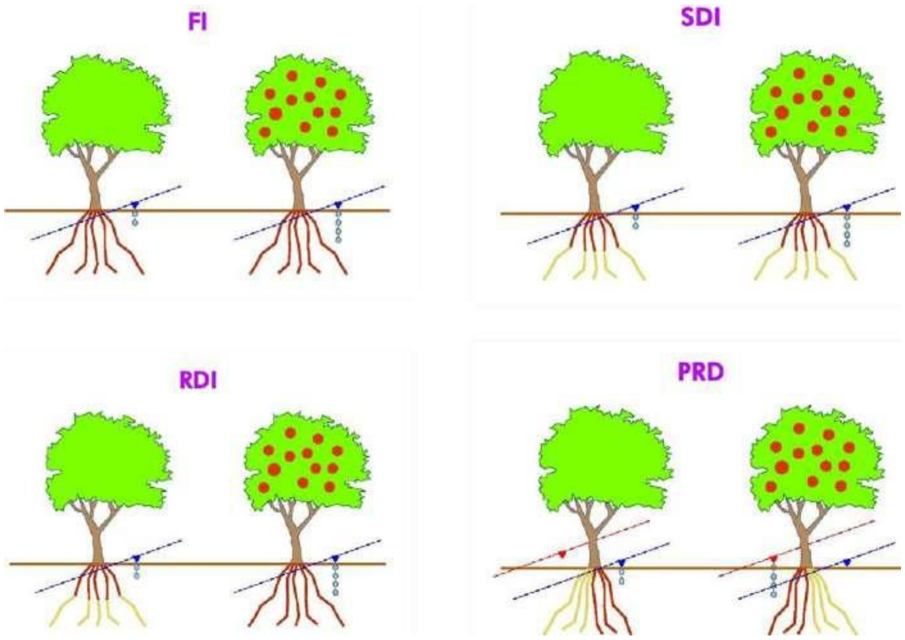

Fig. 1. Graphic pattern of full irrigation (FI), sustained deficit irrigation (SDI), regulated deficit irrigation (RDI) and partial root drying (PRD) strategies in fruit trees.

Domingo et al., (1996) showed that the main critical period corresponds to the rapid fruit growth phase, when water deficit causes a delay in attaining marketable fruit size, whereas moderate water deficit applied during flowering-fruit set-fruit cell division period is not critical in terms of yield. In fact, the effect of water deficit applied during this last phenological period on yield is related not only with the water deficit level achieved but also with the plant species. In Salustiana orange trees (*Citrus sinensis* (L.) Osbeck) on sour orange rootstock (*Citrus aurantium* L.), Castel and Buj (1990) attained a decrease in yield of only 4%, whereas Ginestar and Castel (1996) observed that Clementina de Nules (*Citrus clementina* Hort ex Tan) on Carrizo citrange (*Citrus sinensis* Osb. *Poncirus trifoliata* (L.) Raf.) were extremely sensitive to water restrictions (yield decrease) during this period.

In very early maturing fruit trees, with a very short period from fruit set to harvest and a very long post-harvest phenological period, deficit irrigation should be applied only during the post- harvest period even though avoiding affect bud induction and floral differentiation processes (Torrecillas et al., 2000; Conejero et al., 2011).

Taking into consideration that the effects of water deficit depend not only on the timing but also on the duration and magnitude of the same, the plant water status during non-critical periods has to be maintained within certain levels of water deficit in order to prevent a moderate, potentially beneficial, drought stress from becoming too severe and ending in reduced yield (Table 2) (Johnson et al., 1992; Kang and Zhang, 2004). In this sense, problems have been found in maintaining a certain level of plant water deficit because, when low amounts of irrigation water are applied, adverse situations such as a sudden increase in temperature may result in severe losses of yield and quality, (Table 2) (Jones, 2004). Other problems have been found when applying RDI in heavy and deep soils because soil water depletion and refill frequently take too long (Table 2) (Girona et al., 1993). Under this situation, the success of RDI depends strongly on the appropriate use of microirrigation techniques and sensors able to provide real time information on soil and plant water status (Dichio et al., 2007; Ortuño et al., 2009). In recent years, the use of plant-based water status indicators has become very popular for planning more precise irrigation programmes, because it is recognized that the tree itself is thebest indicator of its water status (Table 1) (Shackel et al., 1997; García-Orellana et al., 2007; Fernandez and Cuevas, 2010). In this sense, sensors like linear variable displacement transducers (LVDTs) are able to measure daily trunk diameter fluctuations (TDF) with great precision, generating sensitive parameters which strongly correlate with established plant water status parameters (Fernandez and Cuevas, 2010; Ortuño et al., 2010). The most common and useful TDF parameters for the irrigation scheduling of woody crops are maximum daily trunk shrinkage (MDS) and trunk growth rate (TGR) (Ortuño et al., 2010; Moriana et al., 2013). Moreover, the operational advantages of TDF measurements in adult trees, such as the possibility of connecting remotely operated irrigation automatic devices, and the ability to rapidly adjust schedules in response to the daily signal, make them very suitable tools for precise RDI scheduling (Conejero et al., 2011; Girón et al., 2015).

2.3. *Partial root drying (PRD)*

This DI strategy, which has also been called partial root-zone irrigation, can be applied through alternate furrow irrigation (Grimes et al., 1968) and by surface and subsurface drip irrigation (Table 1) (Samadi and Sepaskhah, 1984), and is based on irrigating only one part of the root zone, leaving another part to dry to a certain soil water content before rewetting by shifting irrigation to the dry side (Dry and Loveys, 1998; Sepaskhah and Ahmadi, 2010) (Fig. 1).

The strategy is based in the idea that, in PRD, roots sense soil drying, triggering the synthesis of the plant hormone abscisic acid (ABA), which reduces leaf expansion and stomatal conductance, while, simultaneously, the roots of the watered side of the soil absorb sufficient water to maintain a favourable plant water status (Table 1) (Liu et al., 2006a; Zegbe et al., 2006; Ahmadi et al., 2010). In addition, other complementary physiological responses to PRD can favour stomatal closure such as lower cytokine levels (Stoll et al., 2000; Davies et al., 2005) and higher xylem pH (Davies and Zhang, 1991; Stoll et al., 2000). Other results in grapevine (*Vitis vinifera* L.) indicated that PRD may also increase root growth (Dry et al., 2000). Currently, no definitive solid criteria exists for deciding the optimum timing of irrigation for each side (Table 2), probably due to the diversity of factors involved, such as evaporative demand, soil characteristics, soil water status at any precise moment, crop phenological stage, etc., any of which may determine the plant response to wetting or drying of each side of

(Saeed et al., 2008). In this sense, the time when soil water extraction from the dry side is negligible has been proposed as the optimum time to switch wetting from the irrigated root side to the non-irrigated side (Kriedmann and Goodwin, 2003). Also, the threshold soil water content at which the maximum xylem ABA concentration is produced was proposed by Liu et al. (2008) as a criterion for switching irrigation.

Some authors showed that crops under PRD gave better yields than the same crops under DI when the same amount of water is applied. This resulted in higher WP and even better fruit quality (Kriedmann and Goodwin, 2003; Kang and Zhang, 2004; Liu et al., 2006a,b). However, Wakrim et al. (2005) reported no significant difference in water use efficiencies (WUE) between PRD and DI, but a substantial increase in WUE when PRD was compared with FI.

3. Emerging fruit crops response to deficit irrigation

*3.1. Jujube (*Zizyphus jujuba *Mill.)*

Jujube tree (family Rhamnaceae) is native to China, where it has been cultivated for more than 5000 years, and to neighbouring areas of Mongolia and the Central Asian Republics. With time, its cultivation has spread to other regions of the world, including to Mediterranean countries. Jujube fruit is an integral part of the culture and way of life of millions of people and has also become important for many regions of the world following its introduction (Azam-Ali et al., 2006); indeed, it can be considered a so-called functional food, since it has nutritional as well as medicinal uses (Choi et al., 2011). Nevertheless, until now jujube has been considered of minor importance and, from a research and development point of view, it has received little attention from most governments.

Jujube is able to withstand severe drought during the growing season (Fig. 2A) and to tolerate very low winter temperatures during its dormancy (Dahiya et al., 1981; Ming and Sun, 1986; Ming and Sun, 1986). In this sense, jujube trees are able to maintain leaf turgor under severe water deficit ($\Psi_{stem} < 3.0$ MPa), essentially by developing two complementary mechanisms – leaf active osmoregulation (stress tolerance mechanism) and the control of water loss via transpiration (stress avoidance mechanism), while allowing substantial gas exchange rates and, as a consequence, good leaf productivity (Ma et al., 2007; Cruz et al., 2012; Galindo et al., 2016). The gradual recovery of leaf conductance after re- watering previously stressed plants can also be considered as a mechanism for promoting leaf rehydration (Cruz et al., 2012). Moreover, the high leaf relative apoplastic water content (RWC_a) levels and the possibility of increasing the accumulation of water in the apoplasm in response to water stress supports a steeper gradient in the water potential between the leaf and soil (Cruz et al., 2012).

Galindo et al. (2016) showed that in contrast with the axiom that expansive cell growth requires the presence of cell turgor, no direct relation between turgor and growth rate exists in jujube fruits. This could be due to an enhancement of a cell elasticity mechanism (elastic adjustment), which would maintain fruit turgor even at severe water stress levels by reducing fruit cell size, or to the fact that jujube fruit growth depends on fruit growth-effective turgor rather than just on turgor pressure. These authors also reported that during most of the fruit ripening stage water can enter the fruits via the phloem rather than via the xylem. This could be related with the increase in sensitivity to drought during this phenological period, when moderate and severe water deficits induce a significant reduction in total marketable fruit yield (number of fruits and/or average fruit weight). In contrast with this last idea, Cui et al. (2008, 2009) concluded that the jujube fruit maturation stage is the optimal stage to implement water deficit strategies and that while water deficit during the fruit growth slightly reduced the growth rate, re-watering had an over-compensatory effect, thus reducing the negative influence on fruit size.

The same authors (Cui et al., 2008, 2009) mentioned the relatively low water requirements of around 360 mm and showed that jujube fruit maturation can be advanced and the fruit yield and quality enhanced if appropriate RDI is applied at certain growth stages (bud burst to leafing and fruit maturation). Also, Gao et al. (2014) showed that jujube fruit responded positively to irrigation practices, the concentration of some taste-related (e.g. glucose, fructose, TSS and malic acid) and health-related (e.g. catechin and epicatechin) compounds being generally much higher in drip irrigated fruits. In this sense too, Collado-González et al. (2013) demonstrated that water deficit did not affect the tendency of procyanidins to self-aggregate but increased the con- tent of procyanidins of low molecular mass (Table 3), improving their potential bioavailability and possible physiological effects on human health. The procyanidin content of fruit from well-watered trees increased during domestic cold storage, whereas the fruits from trees suffering severe water stress lost some of their procyanidin content. Moreover, in a subsequent paper, Collado-González et al. (2014) pointed to a certain proportionality in the response of jujube fruits to moderate and severe deficit irrigation during fruit maturation. So, when plants were exposed to moderate water deficit (Ψ_{stem} from 1.40 to 2.28 MPa) during this phenological period there was no change in fruit size, moisture content, firm- ness, or fruit peel and flesh colour compared with fully irrigated trees. Only when a more severe water stress (Ψ_{stem} from 1.40 to 3.14 MPa) was reached, there were significant increases in the sucrose and arabinose contents measured (Table 3). In addition, the response of fruit amino acids to water deficit was not as sensitive as expected, since there was no direct relationship with the magnitude of the water deficit. However, the decrease in fruit asparagine content as a result of severer water deficit is a positive aspect, because this amino acid is the major precursor of acrylamide, a potentially toxic compound formed during the heat-processing of some plant foods. However, severe water deficit produced smaller fruit, with a lower moisture content and yield, accompanied by changes in firmness and peel and flesh colour.

*3.2. Loquat (*Eriobotrya japonica *Lindl.)*

Loquat is a subtropical evergreen tree that belongs to the family Rosaceae, subtribe Pyrinae (formerly subfamily Maloideae) (Potter et al., 2007). Some of the common names of loquat include Japanese plum, Japanese medlar, Maltese plum, etc. It is considered indigenous to southeastern China and possibly southern Japan, because it is said to have been cultivated there for over 1000 years. Actually, more than 30 countries in subtropical and mild-temperate regions of the world are cultivating selections of loquat cultivars performed during the 19th century (Feng et al., 2007; Ferreres et al., 2009; He et al., 2011).

It is important to point out that loquat is characterized by an unusual phenology that makes it different of the traditional temperate fruit crops. It blooms in autumn on apical panicles formed on current year wood, developing fruits during winter and ripening in early spring (Fig. 2B). Moreover, this fruit as

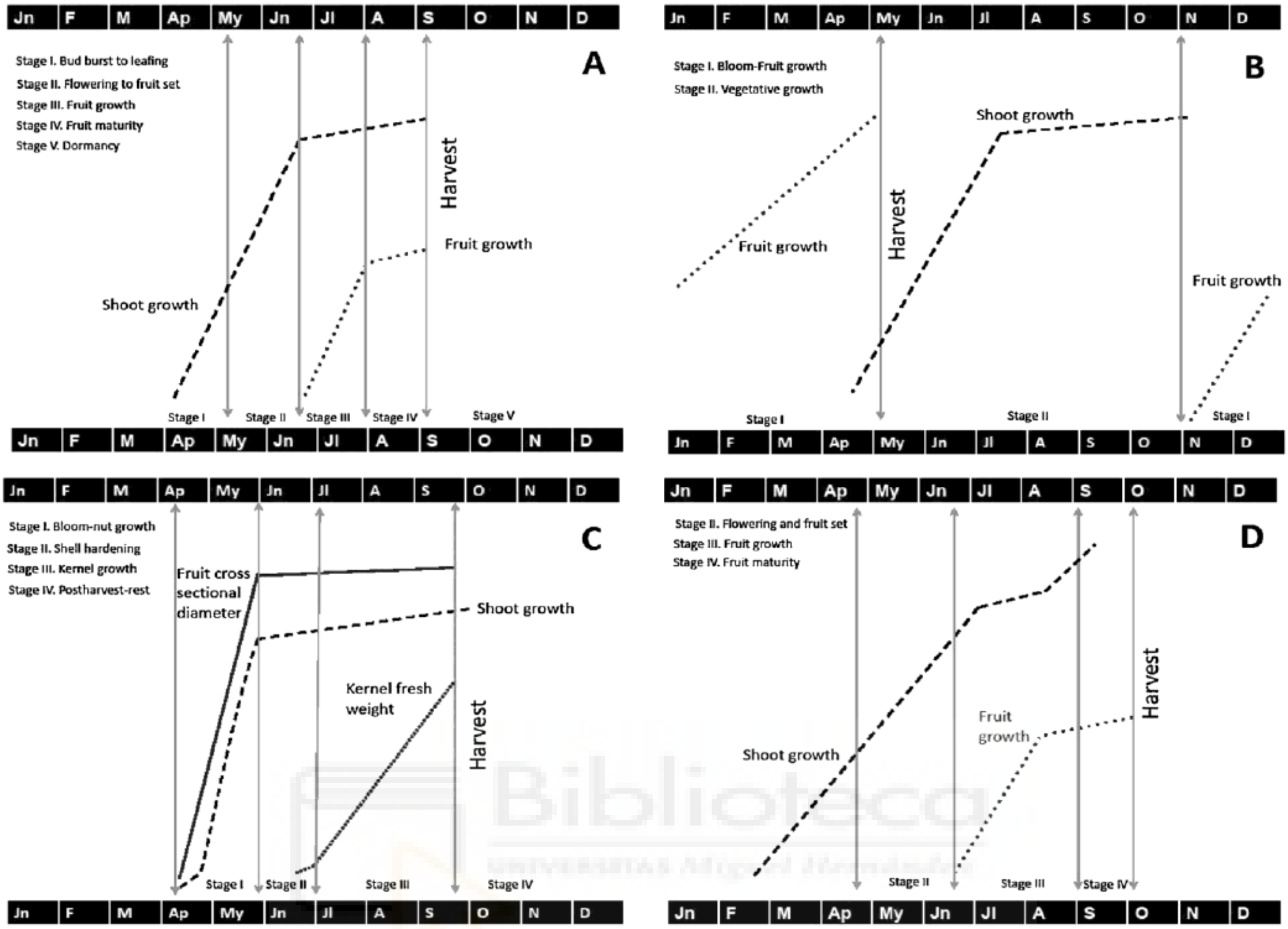

Fig. 2. Seasonal pattern of fruit and shoot growth of jujube (*Z. jujuba*, cv Grande de Albatera) (A), loquat (*E. japonica*, cv Algerie) (B), pistachio (*P. vera*, cv Kerman) (C) and pomegranate (*P. granatum*, cv Mollar de Elche) (D) plants in the southeast (A, B and D) and central (C) Spain conditions. Sources: Hernández et al. (2015), Cuevas et al. (2007a), Memmi et al. (2016b) and Melgarejo et al. (1997), respectively.

other pomes presents a sigmoidal pattern of fruit growth (Dennis, 1988; Cuevas et al., 2003) and arrives at markets before any other spring fruit (Cuevas et al., 2007a; Hueso and Cuevas, 2008).

Research on mechanisms developed by loquat plant to resist drought is very scarce, mainly at plant water relations levels. Diurnal and seasonal gas exchange values in loquat plants respond to changes in plant water status and to changes in evaporative demand, showing minimum values in summer. Moreover, the diurnal trend of photosynthetic rate in loquat, at least during autumn and winter, was characterized by a double-picked curve, suggesting the predominance of genotype over the environmental factors on the loquat gas exchange behaviour (Stellfeldt et al., 2011), because this last behaviour diverges from that indicated for woody Mediterranean vegetation, which is characterized by a maximum value in the morning, declining towards midday, and remaining more or less constant afterward. Recently, Zhang et al. (2015) observed some loquat drought stress tolerance mechanisms: i) the increase in chlorophyll content, which can enhances photosynthesis under water deficit, ii) the increase in the content of soluble sugars and proline of roots, which increased the osmotic adjustment and the favourable water potential gradient for water into the roots, iii) the increase in the ABA content of leaves, which induced the stomata closing and improved the water-use efficiency, and iv) the increase in the levels of antioxidant enzyme activities mainly at leaf level. The ability of loquat plants to develop leaf active osmoregulation was earlier suggested by García-Legaz et al. (2005) who studied the effect of salinity on the water relations of loquat plants on two different rootstocks. Luo et al. (2007) studied the response of two different loquat cultivars to water deficit and concluded that 'Changhong No. 3', the more water deficit resistant cultivar, responds to water deficit with a higher increase in stomatal density and reducing stomatal size than 'Jiefangzhong' cultivar. In addition, in 'Jiefangzhong' cultivar, leaf photosynthetic pigment concentrations decrease in response to drought stress, while in 'Changhong No. 3' the concentrations of photosynthetic pigments increased markedly under light drought stress.

Hueso and Cuevas (2008) estimated relatively high loquat water needs of around 724 mm, and demonstrated observing the long term response of this crop to postharvest RDI that this crop can be considered as a model for the continuous

Table 3
Effect of different deficit irrigation (DI) strategies (SDI, sustained deficit irrigation; RDI, regulated deficit irrigation) on health-related compounds content (↑, increased; ↓, decreased; ≈, no affected) in the edible portion of jujube, pistachio and pomegranate fruits with a non-exhaustive list of references.

Fruit Crop	DI strategy	Compound		Response to moderate water deficit	Response to severe water deficit	References
Jujube (Ziziphus jujubaMill.)	SDI/RDI	Epicatechin		↑	↑	Collado-González et al. (2013)
		Total B type procyanidins		↑	↑	
		Self-aggregated procyanidins		≈	≈	
	SDI/RDI	Vitamin C		≈	↑	Collado-González et al. (2014)
		Sugars	Sucrose or arabinose	↑	↑	
			Glucose	↑	↑	
		Organic acids	Malic or oxalic	↑	↑	
			Citric	≈	↓	
		Proline		≈	↑	
		Asparagine		≈	↓	
		Other amino acids		No uniform behaviour	No uniform behaviour	
	SDI	Flavonoids	Epicatechin or catechin	↓	↓	Gao et al. (2014)
			Procyanidins	≈	≈	
			Rutin	↓	↑	
			Quercetin	↑	≈	
		Total phenolic compounds		≈	≈	
		Sugars (sucrose, glucose or fructose)		↓	↓	
		Organic acids (malic, succinic or citric)		↓	↓	
		Ascorbic acid		≈	≈	
Pistachio (Pistacia veraL.)	RDI	Fatty acids	Oleic or palmitic	≈	≈	Carbonell-Barrachina et al. (2014)
			Linoleic	↑	↑	
		Volatile compounds	Aldehydes	↓	↑	
			Pyrazines and terpenes	↑	↓	
Pomegranate (Punica granatumL.)	SDI	Anthocyanins		≈	↓	Mena et al. (2013)
		Phenolic compounds		↓	↓	
		Punicalagin		↓	↓	
		Ellagic acid		≈	≈	

application of RDI strategies, mainly for the economic benefits of saving water during summer, increasing fruit size and grading and fruit value and gross revenue without affecting yield (Hueso and Cuevas, 2008; Cuevas et al., 2009). This positive response of loquat to RDI is based in two main facts; the clear separation between vegetative and reproductive growth, allowing the application of postharvest RDI without affecting fruit growth, and the improving of fruit value when postharvest RDI is applied because important advancing harvest time in the next season can be achieved. In this sense, the most profitable RDI strategy is the complete suppression of watering from around one month after the end of previous harvest (early June) up to reach a Ψ_{stem} value circa – 2.2 MPa (8–9 weeks), because do not alter the formation of the floral organs and increase the advancement of bloom next season (Cuevas et al., 2007a,b, 2009, 2012), while prolonging the water deficit period during one additional month (August) may impair flower development in loquat (Rodríguez et al., 2007).

Due to the fact that research on loquat response to RDI has been always focusses on fruit earliness due to its enormous importance in loquat price and commercialization and the high susceptibility of mature loquat fruits to mechanical damage during harvest and postharvest handling, to the best of our knowledge, do not exist publications on the effect of deficit irrigation on loquat quality. Since loquat is a non-climacteric fruit, premature picking is inadvisable because fruits are excessively acid and taste unpalatable to consumers. Thus, research has been focussed to stablish fruit maturity indices in order to optimize harvesting. Pinillos et al. (2011) suggested that at every picking date, only those fruits with a skin colour that corresponds to a minimum TSS and TSS/TA values should be harvested, especially at the earliest harvests of the season. Also, a TSS/TA of 0.7 was previously proposed as minimum value for harvest (Pinillos et al., 2007). Recently, Cañete et al. (2015) showed the consumers preference for light orange skin fruits rather than fully ripe ones due to their greater firmness, fewer skin defects and better balance between sweetness and acidity, and proposed harvesting loquat fruits with a minimum value of TSS of 10 Brix and a TSS/TA ratio close to 1.0 to guarantee eating quality and consumer satisfaction.

3.3. *Pistachio (*Pistacia vera *L.)*

Pistachio tree is native to western Asia and Asia Minor, from Syria to the Caucasus and Afghanistan. They are mentioned in the Old Testament. Archaeological evidence from Turkey indicates that the nuts were being used for food as early as 7000 B.C. Pistachio is a member of the Anacardiaceae or cashew family, and is the only commercially edible nut among the eleven species in the genus *Pistacia* and by far the most economically important. The pistachio was introduced into Italy from Syria early in the first century A.D., and subsequently its cultivation spread to other Mediterranean countries.

Pistachio trees are considered one of the most drought resistant fruit species, because they can survive under extreme drought conditions. Spiegel-Roy et al. (1977) observed that under desert conditions pistachio trees were able to differentiate sufficient flower buds to provide an appreciable yield and that roots were uniformly spread down to a depth of 2.40 m even if soil moisture in all the horizons was below the permanent wilting point of soil. Related with this last characteristic, some authors, e.g. Lin et al. (1984) and Germana (1997), suggested that pistachio drought resistance mainly depends on extensive root system development, because, despite it commonly being though a xerophyte, it does not present the morphological characteristic of such in the leaves, showing, instead, high values of net photosynthesis (Pn) and leaf conductance (g_{leaf}). Furthermore, the leaves can be considered as *isolaterals* since their upper and lower pages are structurally similar, with almost identical stomatal density and conductance. Also, Kanber et al. (1993) showed that root activity is confined to shallower soil depths in short interval irrigation conditions. Another singularity of pistachio trees is that both yield and the water stress level regulate the flower bud drop that occurs before the beginning of kernel growth. So, the following year's pistachio yield can decrease considerably as a consequence of a higher percentage of flower buds dropped i) in years of higher yield or ii) when a severe water deficit during fruit stage II takes place (Pérez-López et al., 2017).

Pistachio plants exposed to water stress also develop stress avoidance and stress tolerance mechanisms. As regard the first sort of mechanism, during pistachio fruit stages I and II (Fig. 2C), when the soil water content is quite high and the evaporative demand of the atmosphere is low, these plants show higher Pn and g_{leaf} values. In contrast, during fruit stage III, at which the evaporative demand of the atmosphere is higher, the pistachio plants show lower Pn and g_{leaf} values (n et al., 2011,b; Memmi et al., 2016a,b). When plants are under water deficit, g_{leaf} values decrease in order to limit water loss through transpiration, and at very pronounced levels of water deficit, the daily pattern of g_{leaf} is modified, showing maximum values in the early morning and decreasing gradually, whereas Pn values remain fairly constant until sunset because this parameter is less sensitive to water deficit than g_{leaf} (D. Pérez- López, unpublished data). In this respect, Behboudian et al. (1986) established that pistachio plants are able to continue their photo- synthetic activity even when Ψ_{leaf} reaches extremely low values of 5.0 to 6.0 MPa. Moreover, this crop has an outstanding capability for leaf thermoregulation, even at sever water stress levels, because pistachio canopies can transpire water at rates far higher than those normally found in mesophytes, and are able to rapidly compensate water losses without showing visible stress condition symptoms (Germana, 1997). In addition, when previously water stressed plants are rewatered, the gradual and slow recovery of the plant water status observed can be considered as a mechanism for promoting leaf rehydration (Memmi et al., 2016b). As regard the development of stress tolerance mechanisms, Gijón et al. (2011) identified changes in the leaf bulk modulus of elasticity during pit hardening (stage II) and active osmotic adjustment at any phenological period. Similarly, Behboudian et al. (1986) showed that pistachio plants at a Ψ_{leaf} value of 6.0 MPa exhibited very high Ψ_P values (3.0 MPa).

Pistachio's water relations are significantly affected by rootstock. According to Gijón et al. (2010), the hybrid from crossbreeding *P. atlantica* Desf. x *P. vera* L. may be the best rootstock for adequately irrigated pistachios since it induces the highest leaf conductance and vigour, whereas in rainfed or deficit irrigated conditions, *P. terebinthus* might be a good choice for its drought tolerance, as it is able to maintain a greater leaf area than non-stressed plants with lower Ψ_{stem} and g_{leaf} values. However, in contrast with these results and the widespread belief, Memmi et al. (2016b) suggested that *P. atlantica* could be a suitable rootstock for deficit irrigated plants.

Because of its reputation for being very resistant to water stress, pistachio is mainly cultivated worldwide under rain-fed conditions. Despite the good crop performance under these dryland conditions, there is a clear tendency to increase the area dedicated to irrigation because the benefits derived from irrigation in this crop are probably higher than in other crops. Irrigation increases yield, nut size and splitting, reduces the alternate bearing pattern and incidence of blank nuts, but has no effect on the hull to kernel ratio (Monastra et al., 1998; Ak and Agackesen, 2006). Sedaghati and Alipour (2006) suggested that early hull splitting, a process that decreases the quality of the yield because the kernel is exposed to invasion by fungi and insects, is related with plant water status from late April to early June. However, Gijón et al. (2009) suggested that early splitting incidence is not related to plant water status but to temperatures below 13 °C.

Pistachio's irrigation water requirements are quite high, varying from 547 to 600 mm when calculated according to Memmi et al. (2016b) or Kermani and Salehi (2006) to 842–1000 mm when calculated according to Testi et al. (2008) or Goldhamer (1995). Taking into account that water is a scarce resource and in future only the most efficient agricultural systems will receive inputs of irrigation water (Fereres et al., 2003), studies into optimizing pistachio deficit irrigation strategies are in progress. For example, Memmi et al. (2014) studied the pistachio response to different levels of water deficit and time of application, concluding that irrigation when kernel weight is increasing (stage III) results in a higher fruit size than when the same amount of irrigation water is distributed between stages I (rapid nut growth) and III. Moreover, these authors showed that shell hardening (stage II) starts when the fruit reaches its maximum external diameter and finishes a short time before the kernel reach its final weight, both processes being simultaneous at the end of hardening and beginning of kernel growth.

Gijón et al. (2009) showed that SDI provided at 50 and 65% of the fully irrigated trees during the growing season reduced total yield and kernel size, even though differences in kernel dry weight were unaffected. Memmi et al. (2016b) showed that RDI during stage II or postharvest does not reduce yield even though it may reduce tree vegetative growth. These authors also indicated that full irrigation and RDI in pistachio trees growing in shallow soils can be successfully scheduled using Ψ_{stem} measurements. Hence, RDI using a Ψ_{stem} threshold value of 1.5 MPa during stage II induced similar yield and production values to full irrigated trees, whereas a Ψ_{stem} threshold value of −2.0 MPa resulted in an extensive delay in the recovery of g_{leaf} values, with concomitant negative effects on long-term pistachio production. Guerrero et al. (2005) studied the recovery of pistachio water relations under RDI and concluded that in order to avoid any adverse effect of water deficit during stage III, irrigation should be increased toward the end of stage II or be clearly higher than 100% ETc from the beginning of stage III. Pérez-López et al. (2017) showed that stages I and III are critical because water deficit reduces the quantity and quality of theyield. However, the effects of different water stress levels at each stage have not been sufficiently studied. In this sense, RDI trees (receiving 50% of the water received by control trees during stages I and II, and the same amount of water as control trees during stage III) provided a similar total yield and percentage of split nuts as full irrigated trees and did not show an alternate bearing pattern, even though they received around 20% less water (Gijón et al., 2009).

Okay and Sevin (2011a,b) studied the effect of irrigation on some pistachio fruit characteristics and concluded that differences among cultivars were more significant under non-irrigated conditions. Irrigation increased kernel weight but did not have a significant impact on shell and kernel colours (Guerrero et al., 2005). Carbonell-Barrachina et al. (2014) showed that the more severe the water stress level achieved during stage II, the harder and crunchier the resulting pistachios.

The kernel fatty acid content of pistachio is also affected by plant water status (Okay and Sevin, 2011a), the oleic acid content increas- ing and the linoleic acid content decreasing in fruits of well irrigated trees. In contrast, Carbonell-Barrachina et al. (2014) indicated that the fatty acid profile of pistachios is dominated by three main com- pounds: oleic acid (50%), linoleic acid (33%), and palmitic acid (13%) and showed (Table 3) that moderate RDI during stage II significantly increased the oil content of the nuts, whereas more severe RDI reduced the oil content, inducing in both cases a significant increase in the content of linoleic acid, which is an essential fatty acid for humans. These authors also studied the effect of RDI on pistachio volatile compounds and concluded that severe RDI during stage II increased the contents of aldehydes (associated with green and vegetable notes) and reduce those of pyrazines (nut and toasted notes) and terpenes (citric notes) (Table 3).

A descriptive analysis of pistachios showed that moderate RDI during stage II leads to an intense “green pistachio” colour, accompanied by higher intensities of nutty and pistachio notes in harder, crunchier nuts with a longer aftertaste. Also, an international consumer study about the opinion of European consumers on pistachios grown under RDI indicated that the kernels resulting from moderate RDI applied during stage II obtained a higher intensities of characteristic sensory attributes and a greater level of satisfaction among international consumers than kernels from FI trees or from those exposed to severe RDI during stage II (Carbonell-Barrachina et al., 2014; Noguera-Artiaga et al., 2016).

*3.4. Pomegranate (*Punica granatum *L.)*

Pomegranate, one of the oldest known edible fruits and one of the seven kinds of fruit mentioned in the Bible, is mainly grown in semi-arid mild-temperate to subtropical climates (Blumenfeld et al., 2000). This species and *Punica protopunica* are the two species that make up the Punicaceae family. *P. granatum* is believed to be a native to the southern Caspian belt (Iran) and northern Turkey, whereas *P. protopunica* is generally accepted as being endemic of the Socotra Island (Yemen) (Janick, 2007).

Pomegranate is considered to be a drought-resistant crop because it supports heat and thrives in arid and semiarid areas, even under desert conditions (Aseri et al., 2008), the mechanisms developed by this crop to confront water stress being mainly stress avoidance and stress tolerance (Rodríguez et al., 2012). More precisely, from the beginning of water deficit conditions, leaf conductance decreases in order to control water loss via transpiration and to avoid leaf turgor loss (stress avoidance mechanism) and when sever water stress levels are reached, active osmotic adjustment is triggered, contributing to the maintenance of leaf turgor (stress tolerance mechanism). Other drought tolerance characteristics commonly seen in xeromorphic plants can be also observed in pomegranate, such as a high relative apoplastic water content (42–58%), which would contribute to the retention of water at low leaf water potentials (Rodríguez et al., 2012).

Despite its good resistance to drought, pomegranate for commercial production requires regular irrigation throughout the season, especially when it is cultivated in arid and semiarid areas, to reduce the incidence of fruit physiopathies (e.g. fruit splitting) (Galindo et al., 2014b; Rodríguez et al., 2017) and to reach optimal growth, yield and fruit quality (Levin, 2006; Holland et al., 2009). In this sense, the period corresponding to the end of pomegranate fruit growth and ripening is clearly critical for the incidence of fruit split- ting. Galindo et al. (2014b) showed that at very severe water deficit levels, despite leaf turgor being maintained, fruit turgor is lost inducing a reduction in fruit expansion. Then, when an important rainfall event takes place, previously water stressed pomegranate fruits are rehydrated asymmetrically because aril turgor increases to a much greater extent than peel turgor, the pressure of the arils on the peel favouring splitting.

Intrigliolo et al. (2013) estimated pomegranate evapotranspiration to be around 412–514 mm, but reports on the effect of irrigation management on pomegranate fruit yield and quality are relatively scarce. The first results indicated that it is possible to control the desired ripening time in pomegranates by applying different irrigation regimes (Sonawane and Desai, 1989). Recently, Galindo et al. (2014a) indicated that SDI applied throughout the pomegranate season to achieve pronounced water deficit levels reduces total yield per tree, the number of fruits per tree and the size of the fruits; however, such a strategy can bring forward the availability of fruits resulting from late flowerings, which, despite their smaller size, are of great interest for the pomegranate trans- formation industry due to their very high content of bioactive compounds. In contrast, other studies mention ambiguous results concerning the effect of SDI on the chemical characteristics of pomegranate fruit. In this sense, Mellisho et al. (2012) concluded that SDI, under moderate water stress, produced some changes in colour and chemical characteristics, which reflected earlier ripening. However, Mena et al. (2013) indicated that pomegranate juice from trees submitted to SDI regimes that produce severe water stress levels was of lower quality and less healthy than the juice from fully irrigated trees. This reduction in quality was due to the fact that the water stress levels caused a dramatic decrease in bioactive phenolic compounds, especially anthocyanins and punicalagin (Table 3); besides, the pomegranates were less attractive for consumers due to their pale red colour. On the other hand, Laribi et al. (2013) showed that pomegranates from SDI trees, submitted to mild water stress during flowering and fruit set and more severe water stress during the linear stage of fruit growth and ripening, had a redder peel and higher level of total soluble solids in the juice.

Intrigliolo et al. (2013) and Laribi et al. (2013) studied the pomegranate response to RDI involving irrigation water restrictions during different fruit stages and concluded that the period comprised by flowering and fruit set could be regarded as non- critical from the yield point of view and that irrigation water restriction during pomegranate fruit growth and ripening enhances peel redness and TSS in the juice. However, restricting the irrigation water during the linear fruit growth period increased the concentration of many bioactive compounds in the juice, such as anthocyanins, that are related to health and taste. Recently, Galindo et al. (2017) showed that a short period of irrigation restriction at the end of ripening period brings the harvest time forward, saves irrigation water, enhances the fruit bioactive compounds content (anthocyanins, phenolic compounds, punicalagin and ellagic acid) and increases the price of the fruit without affecting marketable yield and fruit size.

Studies on the response of pomegranate trees to PRD have been

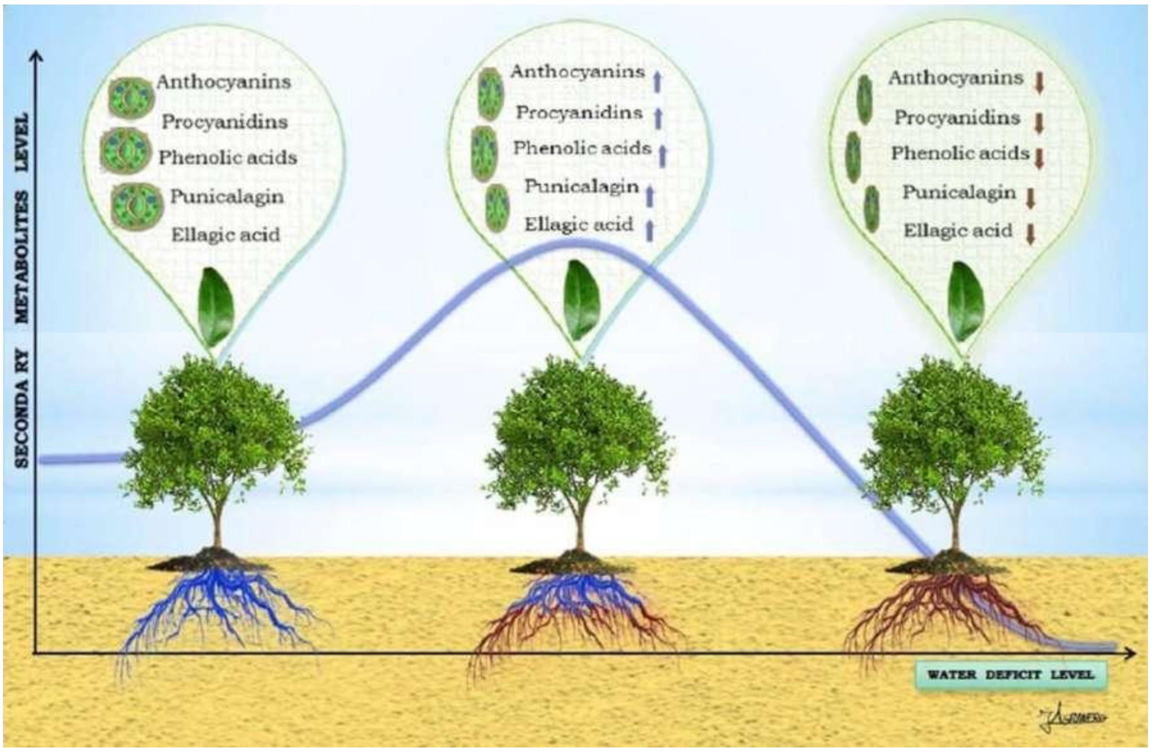

Fig. 3. Quadratic relationship between secondary metabolites content in the fruits and plant water status (blue line) (Horner, 1990). Under mild water deficit, stomatal regulation may leads to a reduction in plant growth, increasing concentration of nonnitrogenous secondary metabolites (central tree). When water deficit increases (right tree), CO_2 assimilation is reduced and carbon is preferentially allocated to the synthesis of primary metabolites, which do not exceeded the amount used for fruit growth to the detriment of the synthesis of carbon-based secondary metabolites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

performed by Parvizi et al. (2014, 2016) and Parvizi and Sepaskhah (2015). These authors compared the following strategies: SDI (50% and 75% of ETc), irrigating only one side of trees (north) through- out the growing season and keeping the other side (south) of the tree dry, PRD (50% and 75% of ETc) and FI, maintaining both sides of the tree wetted. The first authors showed that both SDI strategies and PRD at 50% ETc induced a decrease in pomegranate yield, and recommended PRD (75% ETc) because, in addition to saving water, yield, intrinsic water use efficiency (WUE) and transpiration efficiency increased. As regard pomegranate fruit quality attributes, Parvizi and Sepaskhah (2015) indicated that both PRD strategies increased the pomegranate fruit juice content and maturity index and decreased the titratable acidity values compared with FI fruits, while the response of fruits to both SDI strategies was the opposite of that observed in response to PRD.

Deficit irrigation can be considered as a tool that significantly improves the postharvest performance of pomegranate. Several authors reported that the fruits resulting from SDI and RDI treatments showed better postharvest behaviour than those from FI because of retarded chilling injury incidence (Peña et al., 2013), higher sensory and nutritional quality and longer shelf life (Laribi et al., 2013; Peña et al., 2013; Peña-Estévez et al., 2016). Moreover, in a study of the effect of different irrigation treatments and the efficacy of a vapour treatment (7–10 s at 95 °C) and using NaClO as sanitizing agents on the quality and shelf life of fresh-cut pomegranate arils, Peña-Estévez et al. (2015) observed a synergistic effect of the water deficit treatment and the postharvest thermal treatment. Best results were obtained for arils from pomegranates grown on trees from which irrigation was withheld for 16 or 26 day prior to harvest, for which a shelf life of 18 days at 5 °C was established.

4. Summary, conclusions and future research needs.

Bearing in mind the characteristics of the emerging crops considered in this review, it is clear that they present different mechanisms to confront water deficit situations, and that different levels of resistance are achieved. In this sense, pistachio trees can be considered the most drought resistant because they can survive under very extreme drought conditions. Their water stress resistance is based on morphological characteristics such as a very extensive root system and the development of stress avoidance and tolerance mechanisms. Pomegranate and jujube trees are also able to withstand severe drought during the growing season and the mechanisms developed by these crops to confront drought are also predominantly stress avoidance and stress tolerance mechanisms. In contrast, an entirely different strategy to confront water deficit is shown by loquat. Its (loquat) strategy can be considered as a drought escape mechanism because it is based on an atypical phenology, completely different from that of traditional Mediterranean temperate fruit crops, blooming in autumn, developing fruits during winter and ripening in early spring. So, fruit growth accounts when Mediterranean climate is wetter and evaporative demand of the atmosphere reaches minimum values, thus avoiding the effects of hot and dry summers. The irrigation water requirements of these emerging crops were not related with the resistance to water stress. So, loquat trees presented the highest seasonal ETc, which was slightly higher than that observed in pistachio and pomegranate trees, and clearly higher than

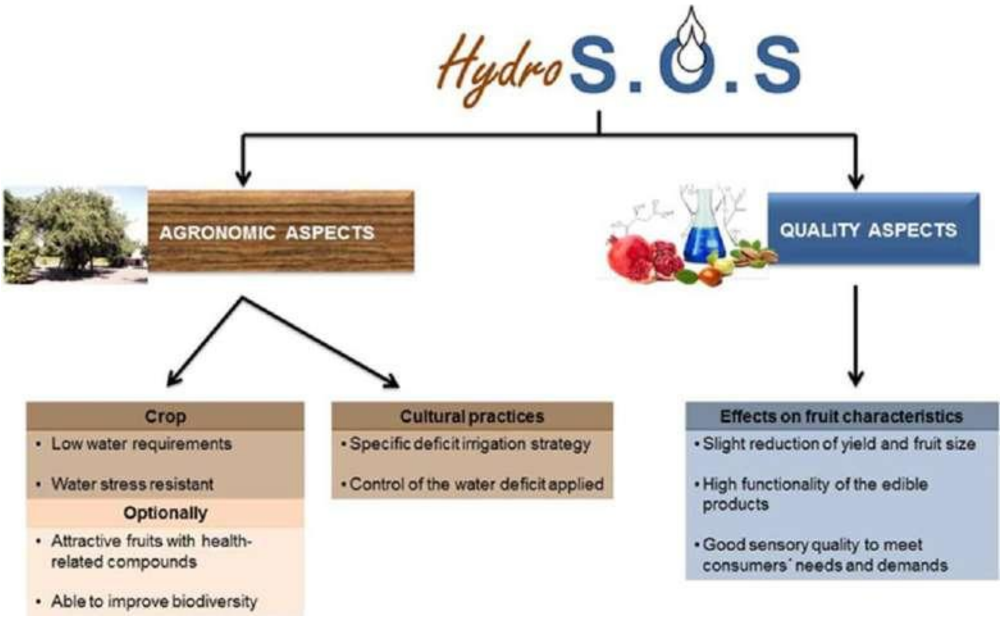

Fig. 4. Main agronomic and fruit quality aspects needed to obtain a *hydroSOS* fruit certification.

that observed in jujube trees. It is clear that loquat can be considered an outstanding crop for its response to the continuous application of RDI, but there are good reasons to conclude that the other emerging crops studied are able to cope with water scarcity due to their positive response to DI strategies, including minimal impact on yields and improved WP. Taking into consideration the effect of DI strategies on fruit quality and the health-related compounds they contain, it is important to underline that research needs to be directed at some very important aspects including: (i) the effect of deficit irrigation on loquat fruit quality, for which, to the best of our knowledge no information exists, and (ii) identifying the optimal water deficit level, its timing and duration for each crop in order to optimize fruit quality and their health-related compounds content. This last consideration is based on the fact that the literature in most cases suggests that fruit quality and the health-related compounds content can be improved by specific DI strategies, but fruit response to moderate and severe water deficit is not proportional in many cases. It is not possible to establish a linear correlation between water stress and some fruit characteristics, especially in the case of some secondary metabolites (Mattson and Haack, 1987; Gobbo-Neto and Lopes, 2007). In an attempt to predict the concentration of phenolic compounds as a function of water status, Horner (1990) proposed a model based on a quadratic relationship between both variables (Fig. 3).When plants are under mild osmotic stress there is a reduction in plant growth and the concentration of non-nitrogenous secondary metabolites increase. When plants are under severe water stress, strong stomatal regulation takes place and CO_2assimilation is much reduced; carbon is preferentially allocated to the synthesis of primary metabolites, which do not exceed the amount used for fruit growth and to the detriment of the synthesis of carbon-based secondary metabolites (Fig. 3).Bearing in mind all the previous considerations, it is evident that farmers who adopt specific DI strategies and cultivate underutilized plant species should be rewarded for (i) making sustainable use of irrigation water, (ii) improving crop biodiversity, (iii) having to accept a slight reduction in their fruit and vegetable yields, and (iv) producing fruits with higher contents of bioactive com-pounds. Fortunately, consumers are willing to pay for special foods, particularly those associated with environmental friendly farming practices that use no chemicals (Martínez-Ruiz and Gómez-Cantó, 2016) – which is the case of the fruits and vegetables grown under DI. However, consumers need to identify such products, which should be clearly labeled and displayed separate from other products of the same type, otherwise their potential will be lost in a sea of products. Very few groups have studied consumer opinion concerning DI fruits (Lopez et al., 2016; Fernandes-Silva et al., 2013),but Noguera-Artiaga et al. (2016) even proposed an identity brand to protect this type of product, which might be called hydroSOStainable or, in abbreviated and easier to remember form, hydroSOS. According to these authors, hydroSOS products will have a solid identity based on two main factors: (i) water deficit can increase the plant secondary metabolite content and, thus, the functionality of the edible products (Ripoll et al., 2014), and (ii) the products are environmentally friendly because of the sustainable use of a very scarce resource, water (Fig. 4).

Noguera-Artiaga et al. (2016) also found that consumers are willing to pay a reasonably higher price for *hydroSOS* pistachios, if they are properly labeled and identified. However, further research is needed to check whether this greater willingness to pay is the similar for all fruits. Finally, it is essential to establish a *hydroSOS* index to certify that the products using the *hydroSOS* logo have been evaluated for their sustainable use of irrigation water and/or their contents of bioactive compounds. This index is under construction and will be based, among other factors, on farmers and traders being able to demonstrate: (i) knowledge of the cultural practices involved, including water management during the non- critical periods, (ii) the timing, level and duration of the applied water deficit, (iii) that suitable monitoring and control of the stress applied has taken place by measuring, for example, the water potential, (v) the precise composition and contents of bioactive compounds, e.g. increased levels of proline (an amino acid used as indicator of plant water stress), and (vi) the good sensory quality of the product in question. If these rules are followed, it should be possible to ensure consumer satisfaction, strengthen their will- ingness to pay a reasonably higher price, and guarantee their future fidelity to these products (Fig. 4). If the index can guarantee all the above, consumer demand will increase, as will the price of *hydroSOS* products and the possible profit for farmers. Hopefully, farmers will become increasingly convinced about the economic benefits of DI and dedicate larger areas to the cultivation of even more crops.

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7.2 Publication 2 (Open Access)

Leaf water relations in *Diospyros kaki* during a mild water deficit exposure

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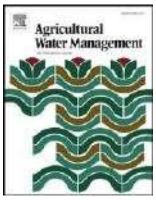

Leaf water relations in *Diospyros kaki* during a mild water deficit exposure

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ABSTRACT

The resistance mechanisms (stress avoidance and stress tolerance) developed by persimmon plants (*Diospyros kaki* L. f. grafted on *Diospyros lotus* L.) in response to mild water stress and the sensitivity of continuously (on a whole-day basis) and discretely (at predawn and midday) measured indicators of the plant water status were investigated in 3-year old 'Rojo Brillante' persimmon plants. Control (T0) plants were drip irrigated in order to maintain soil water content at levels slightly above soil field capacity (102.3% of soil field capacity) and T1 plants were drip irrigated for 33 days in order to maintain the soil water content at around 80% of soil field capacity. The results indicated persimmon plants confront a mild water stress situation by gradually developing stomata control (stress avoidance mechanism) and exhibiting some xeromorphic characteristic such as high leaf relative apoplastic water content, which could contribute to the retention of water at low leaf water potentials. In addition, sap flow measurements made by the heat-pulse technique were seen to be the most suitable method for estimating persimmon water status, because it provided the highest signal intensity (actual value/reference value):noise (coefficient of variation) ratio in almost all intervals of time considered and provides continuous and automated registers of the persimmon water status in real time.

1. Introduction

The decrease in the profitability of some Mediterranean fruit tree industries in recent years has led to the search for other fruit trees as alternatives. This situation has provided very important collateral advantages, including such as the enrichment of biodiversity, which is fundamental for ecosystem functioning, more sustainable agricultural production and increased food and nutritional security (Thrupp, 2000; Toledo and Burlingame, 2006; Chappell and LaValle, 2011). In this sense, persimmon (*Diospyros kaki* L. f.) tree culture in the Spanish Mediterranean basin is steadily increasing, aided by its excellent adaptation to temperate warm climates, high yields, high commercial value of the fruit, and excellent post-harvest storage life. Persimmon is native to the mountains of central China and Japan (Mowat and George, 1994; Llácer and Badenes, 2002; George et al., 1997) and is included in the list of so-called underutilized or minor fruit crop species.

Badal et al. (2010) suggested that the irrigation water requirements of persimmon are quite large. So, besides genetic factors, water deficit is considered as the main environmental factor affecting unstable per-

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simmon fruit production (physiological fruit drop and biennial bearing) (Suzuki et al., 1988; Yamamura et al., 1989; Yakushiji et al., 2013). As a consequence, irrigation may be the main limiting factor for persimmon culture in Mediterranean agrosystems due to the persistent shortage of water resources. For this reason, persimmon irrigation will need to be based on the use of very precise deficit irrigation management strategies that are able to significantly reduce the amount of irrigation water neccesary with minimum effects on yield and fruit quality.

Under deficit irrigation conditions, the continuous and precise control of tree water status is crucial in order to prevent a potentially beneficial water stress from becoming too severe and ending in a reduction in the yield or fruit quality (Johnson and Handley, 2000). In this sense, the use of plant-based water status indicators may be considered as an ideal tool for precise deficit irrigation scheduling in fruit trees, as has been reported by Naor (2000); Lampinen et al. (2001); García-Orellana et al. (2007); Ortuño et al. (2009a, b), Ortuño et al. (2010) and Conejero et al. (2011). A suitable plant-based water stress indicator for use in irrigation scheduling practices has to be sufficiently sensitive, consistent and reliable for detecting minimum changes in the plant water status. Moreover, it is important to consider that the magnitude of any plant-based water status indicator, even in a well-watered tree, is not constant over a period of days with different environmental conditions. Therefore, the absolute values of these indicators, registered without considering the evaporative demand, might be meaningless. For this reason, for irrigation scheduling it is better to use the concept of signal intensity (SI), normalizing the absolute values with respect to values in non-limiting soil water conditions (Fernandez and Cuevas, 2010; Ortuño et al., 2010).

The irrigation protocol for trees using plant-based water status indicators consists of maintaining the plant-based water status indicator SI at around a threshold value, decreasing the irrigation rate when the SI does not exceed the threshold value, and increasing the irrigation rate when the SI exceeds the threshold value. When fruit trees are grown with high frequency irrigation the irrigation water amounts to be applied are usually estimated daily (Conejero et al., 2011), every three days (Conejero et al., 2007) or weekly (Velez et al., 2007).

The discrete measurement of predawn or midday leaf water potential (Ψ_{pd} or Ψ_{md}) and midday (12 h solar time) stem water potential (Ψ_{stem}) are the most widely used approaches for evaluating plant water status (McCutchan and Shackel, 1992; Naor, 2000). However, in recent years the possibility of obtaining real time, continuous and automated registers of the plant water status, avoiding frequent trips to the field and a significant input of manpower, has led to the increased use of alternative indices using plant sensors such as sap flow (SF) and maximum daily trunk shrinkage (MDS), a single parameter obtained from trunk diameter monitoring, which can be used for full and deficit irrigation scheduling in fruit trees (García-Orellana et al., 2007; Ortuño et al., 2009a, b; Conejero et al., 2011; Moriana et al., 2013)

To the best of our knowledge, research on the response, at plant water relations level, of persimmon plants to drought is very scarce. Nevertheless, Yakushiji et al. (2013) showed that predawn leaf turgor potential (Ψ_{ppd}) began to decrease when Ψ_{pd} fell below ca. - 0.7 to - 0.8 MPa, and that the response of fruit water status to drought clearly depends on the fruit growth stage. Yamamura et al. (1989) indicated that even a moderate water deficit (leaf water potential (Ψ_{leaf}) values around - 1.8 MPa) increased fruit drop. Also, Badal et al. (2010) assessed the usefulness of the MDS, as a persimmon water deficit indicator.

Bearing the above in mind, the purpose of the present study was (i) to evaluate the sensitivity of continuous and discretely measured indicators of the plant water status to use in irrigation scheduling in persimmon trees, and (ii) to analyze leaf water relations in order to clarify the response mechanisms (stress avoidance and stress tolerance) developed by persimmon plants in response to mild water stress.

2. Materials and methods

2.1. Experimental conditions, plant material and treatments

The experiment was carried out during the summer of 2016 at a farm located near the city of Murcia (Spain) (38°1′N, -1°3′W). The soil is a Calcaric fluvisol with clay texture. Soil volumetric water contents (θ_v) at saturation, field capacity and permanent wilting point were 0.48, 0.42 and 0.28 m^3 m^{-3}, respectively. The irrigation water had an electrical conductivity of between 1.2 and 1.4 dS/m and a Cl^- concentration ranging from 20 to 35 mg l^{-1}.

The climate of the area is typically Mediterranean, with mild winters, low annual rainfall, and hot dry summers. During the experimental period, average daily maximum and minimum air temperatures were 32 and 19 °C, respectively, the mean daily air vapour pressure deficit (VPD_m) (Allen et al., 1998) ranged from 0.89 to 2.64 kPa, and reference crop evapotranspiration (ETo, Allen et al., 1998) was 171 mm. No rainfall was recorded during the experimental period.

The plant material consisted of 3-year old persimmon trees (*Diospyros kaki* L. f. cv. 'Rojo Brillante' grafted on *Diospyros lotus* L.). Tree spacing followed a 3 m x 5 m pattern. Pest control and fertilization practices were those normally used by the growers, and no weeds were allowed to develop within the orchard.

Two irrigation treatments were considered, in which irrigation was carried out daily and during night time using a drip irrigation system with one lateral pipe per tree row. From day of the year (DOY) 218–251, in order to guarantee non-limiting soil water conditions, control plants (treatment T0) were irrigated using six emitters (each delivering 4 l h^{-1}) per plant in order to maintain soil water content in the 0–60 cm soil depth at levels near constant and slightly above soil field capacity. In the T1 treatment water was applied at 70% of control trees.

2.1.1. Measurements

θ_v was measured with a portable FDR sensor (HH2, ΔT, U.K.) previously calibrated. The measurements were made in four plots per treatment. The access tubes for the FDR sensor were placed in the irrigation line at about 30 cm from an emitter. The data were obtained at 0.10, 0.20, 0.30, 0.40 and 0.60 m depth. Ψ_{leaf} was measured on the south facing side and the middle third of the trees, in two fully developed leaves per tree of each replicate, using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), as recommended by Turner (1988). After measuring Ψ_{leaf}, the leaves were frozen in liquid nitrogen and the osmotic potential was measured after thawing the samples and expressing sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Leaf turgor potential (Ψ_p) values were derived as the difference between osmotic and water potentials. The Ψ_{stem} was measured in a similar number and type of leaves as used for Ψ_{leaf}, enclosing leaves in a small black plastic bag covered with aluminium foil for at least 2 h before measurements in the pressure chamber. Leaf conductance (g_{leaf}) in attached leaves was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of the leaves and in a similar number and type of leaves as used for the Ψ_{leaf} measurements.

At the end of the experimental period, two pressure-volume (PV) curves were performed per replicate in order to determine values of leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at the turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ), relative water content at the turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) (Tyree and Hammel, 1972; Tyree and Richter, 1981, 1982; Savé et al., 1993). For this, leaves were excised at predawn and resaturated by dipping the petioles in distilled water for 24 h in darkness at 4 °C. The resaturated leaves were weighed using an analytical balance (± 0.1 mg precision), placed in the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber). Once depressurized, the leaf was allowed to transpire outside the pressure chamber on the laboratory bench at room temperature (22 ± 2 °C). Leaves were repeatedly weighed and their balance pressures were determined over the full range of the pressure gauge (Kikuta and Richter, 1986). Data for initial saturated weight, intermediate fresh weight (corresponding to values for Ψ_{leaf}), and final dry weight (at 80 °C for 48 h) were used to calculate the relative water content (RWC) (Barrs and Weatherley,1962).

The curves were drawn using a type II transformation (Tyree and Richter, 1982). The reciprocal of Ψ_{leaf} was plotted against RWC, and the resultant relationships displayed both linear and non-linear regions. Extrapolation on the straight portion of the curve obtained for a value of RWC = 1 gave the reciprocal of the Ψ_{os} and extrapolation to the abscissa gave RWC_a. The Ψ_{tlp} and RWC_{tlp} were estimated as the intersection between the linear and curvilinear portions of the PV curve. The ϵ of leaf tissue at 100% RWC (RWC_o) was estimated according to Patakas and Noitsakis (1999) as ϵ (MPa) = $(\Psi_{os} - \Psi_{stlp})\ (100 - RWC_a)/(100 - RWC_{tlp})$, where Ψ_{stlp} is the osmotic potential at the turgor loss point and Ψ_{os} values correspond to those obtained from the analysis of the PV curves.

The micrometric trunk diameter fluctuations (TDF) were measured throughout the experimental period on four trees per treatment, using a set of linear variable displacement transducers (LVDT) (model DF ± 2.5 mm, accuracy ± 10 µm, Solartron Metrology, Bognor Regis, UK) attached to the trunk, with a special bracket made of Invar, an alloy of Ni and Fe with a thermal expansion coefficient close to zero (Katerji et al., 1994), and aluminium. Sensors were placed on the north side, 10 cm above the graft point of each tree, and were covered with silver thermoprotected foil to prevent heating and wetting of the device. Measurements were taken every 10 s and the datalogger (model CR10X, Campbell Scientific, Logan, UT, USA) was programmed to report 15 min means. MDS was calculated as the difference between maximum and minimum daily trunk diameter.

SF was measured using the compensation heat-pulse technique (Swanson and Whitfield, 1981) in the same trees used for TDF measurements throughout the experimental period. One set of heat pulse probes was located above the LVDT sensors on each tree. Each set consisted of a heater needle of 1.8 mm diameter and two temperature probes also of 1.8 mm diameter installed in parallel holes drilled radially in the trunks at 10 mm downstream and 5 mm upstream. Each heat-pulse probe had three thermocouple sensors to monitor the sap velocity at a radial depth of 5, 12 and 21 mm below the cambium. Sap velocity was measured following the procedure of Green et al. (2003), using the theoretical calibrations of Swanson and Whitfield (1981) to account for the probe-induced effects of wounding. The volume fractions of wood and water determined by López-Bernal et al. (2014) were used. The temperature signals and the corresponding heat-pulse velocities were recorded at 30 min intervals using heat-pulse instrumentation controlled by a datalogger (CR10X, Campbell Scientific Ltd., Logan, Utah)

2.1.2. *Statistical design and analysis*

The design of the experiment was completely randomized with four replications, each replication consisting of three adjacent tree rows, each with seven trees. Measurements were taken on the inner tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), whereas the other trees served as border trees. Statistical analysis was performed by an analysis of variance using the general linear model (GLM) of SPSS (SPPS, 2002). Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

In the T0 treatment, θ_v between 0 and 0.60 m in depth was nearly constant and slightly above field capacity (102.3% of θ_v values at field capacity) (Fig. 1). In the T1 treatment, θ_v values decreased achieving constant values of around 81% of soil field capacity from DOY 222–251. Ψ_{pd} values in T0 plants were very high and fairly constant throughout the experimental period, while in T1 plants Ψ_{pd} values were significantly lower than in T0 plants, being characterized by a slight decrease from the beginning of the experiment to DOY 222, when mínimum values were reached (Fig. 2A). Ψ_{md} values in T0 plants during the experimental period were fairly constant and higher than those in T1 plants, whereas in T1 plants the Ψ_{md} values gradually decreased, achieving minimum values on DOY 231 and increasing slightly thereafter (Fig. 2B). Ψ_{ppd} and leaf turgor potential at midday (Ψ_{pmd}) values in both irrigation treatments were always above zero, indicating that turgor was maintained during the experimental period. However, both parameters showed differences in the response to irrigation treatments (Figs. 2C and 2D). The Ψ_{ppd} values in both treatments were high and showed some tendency to fluctuate (Fig. 2C). Furthermore, Ψ_{ppd} values in T1 plants were always lower than in T0 plants. Ψ_{pmd} values in T1 plants were also lower than those in T0 plants, even though Ψ_{pmd} values in T1 plants showed a gradual but clear tendency to decrease during the experimental period, reaching minimum values of 0.36 MPa at the end of the experiment on DOY 251 (Fig. 2D).

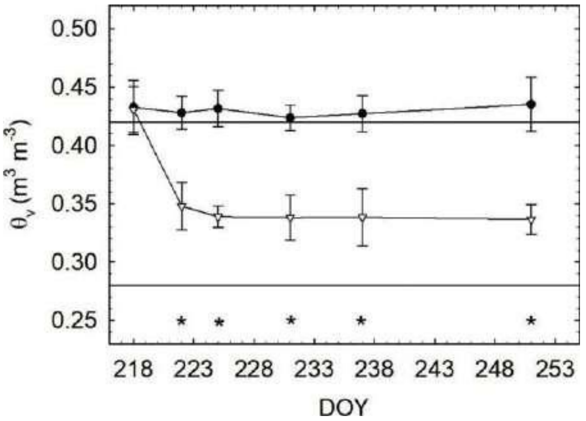

Fig. 1. Soil volumetric water content (θv) to a depth of 0.60 m (mean ± SE) in the T0 (closed circles) and T1 (open triangles) irrigation treatments during the experimental period. The lower horizontal line represents θv at permanent wilting point and the upper horizontal line represents θv at field capacity. Asterisks indicate significant differences at $P \leq 0{\cdot}05$ (n = 4).

Ψ_{stem} values in both irrigation treatments were higher than the corresponding Ψ_{md} values throughout the experimental period and behaved somewhat similarly to Ψ_{pd} values (Figs. 3 A, 2 B and 2 A, respectively). So, Ψ_{stem} values of T0 plants were almost constant during the experimental period while in T1 plants they showed a tendency to decrease, almost all the time with lower values than those observed in T0. The g_{lmd} values in T0 plants were nearly constant during the experimental period, whereas g_{lmd} values in T1 plants were clearly lower than in T0 plants, gradually decreasing during the experimental period (Fig. 3B).

During the experimental period, regardless of the treatment, Ψ_{leaf} values exhibited a similar circadian rhythm on the five measuring dates, reaching maximum values at predawn, decreasing rapidly in the morning and reaching minimum values at around 14.00–17.00 h, after which they gradually recovered (Fig. 4). Differences between the circadian Ψ_{leaf} values of the T0 and T1 varied from day-to-day. At the end of the experimental

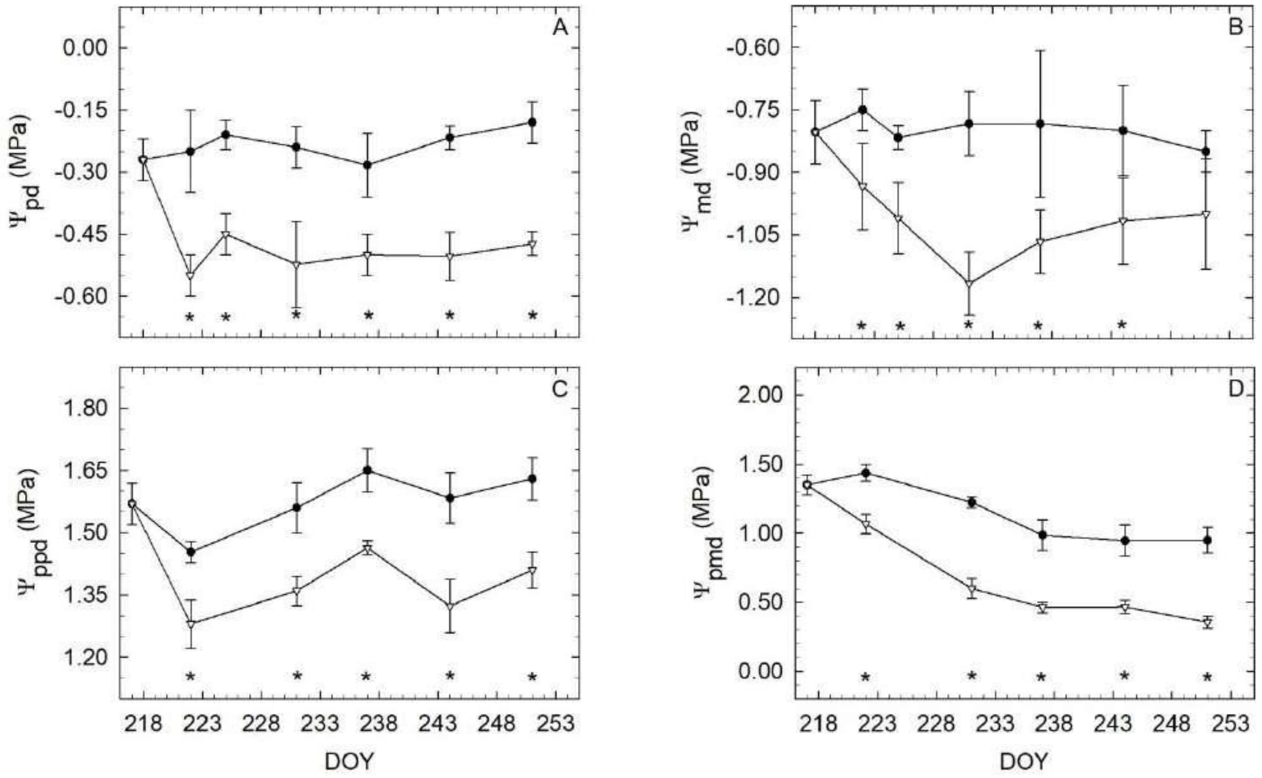

Fig. 2. Predawn leaf water potential (Ψ_{pd}, A), midday leaf water potential (Ψ_{md}, B), predawn leaf turgor potential (Ψ_{ppd}, C) and midday leaf turgor potential (Ψ_{pmd}, D) values for persimmon plants in T0 and T1 treatments during the experimental period. Symbols as in Fig. 1.

period (DOY 251), the daily Ψ_{leaf} pattern in T1 plants was characterized by a gradual decrease, reaching minimum values at 14.00 h and showing only a very slight recovery during the afternoon. At sunrise, the increase in radiation induced stomatal opening while g_{leaf} increased to reach maximum values between 10.00 and 14.00 h, after which it progressively decreased (Fig. 4). T0 plants showed higher g_{leaf} values than those in plants under water deficit (T1) during most of the day and but specially when daily maximum g_{leaf} values were achieved. Differences in g_{leaf} values between T0 and T1 plants gradually increased due to the response of T1 plants to the deficit irrigation, which gradually decreased the duration of maximum stomatal opening. Low and near constant g_{leaf} values were registered during most of the day from DOY 231–251 (Fig. 4). Ψ_p values showed a similar circadian rhythm on the five studied dates, characterized by maximum values at predawn and minimum values at 12.00–17.00 h (Fig. 4). Ψ_p values in T1 plants tended to be lower than in T0 plants, especially in the central hours of the day (12.00–17.00 h).

Daily SF values in T0 plants were characterized by a more pronounced fluctuation than was seen in T1 plants, where they decreased gradually, showing differences between treatments from DOY 221 onwards and remaining almost constant from DOY 223 onwards (Fig. 5A). MDS values in T1 plants were higher than in T0 plants. In addition, differences in MDS values between treatments were significant the day immediately after the beginning of the experiment, 2 days earlier than the differences in SF became evident (Fig. 5A and B). In contrast with the behaviour observed in daily SF values, no differences between treatments were observed in MDS values from DOY 226–230, on DOY 235 and on DOY 238. The regression analysis between SF and Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd}, obtained by pooling data for the whole observation period, demonstrated that decreases in SF values were associated with decreases in Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} values (Fig. 6).

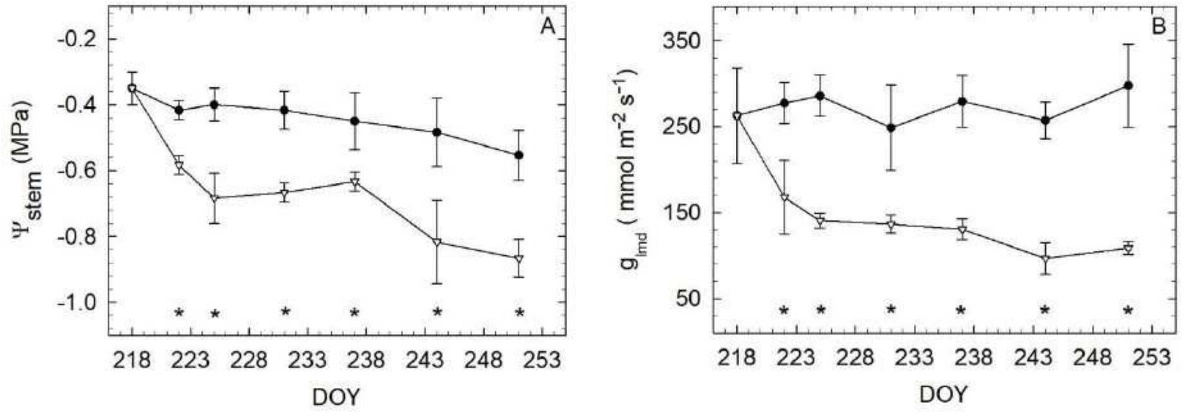

Fig. 3. Midday stem water potential (Ψ_{stem}, A) and midday leaf conductance (g_{lmd}, B) values for persimmon plants in T0 and T1 treatments during the experimental period. Symbols as in Fig. 1.

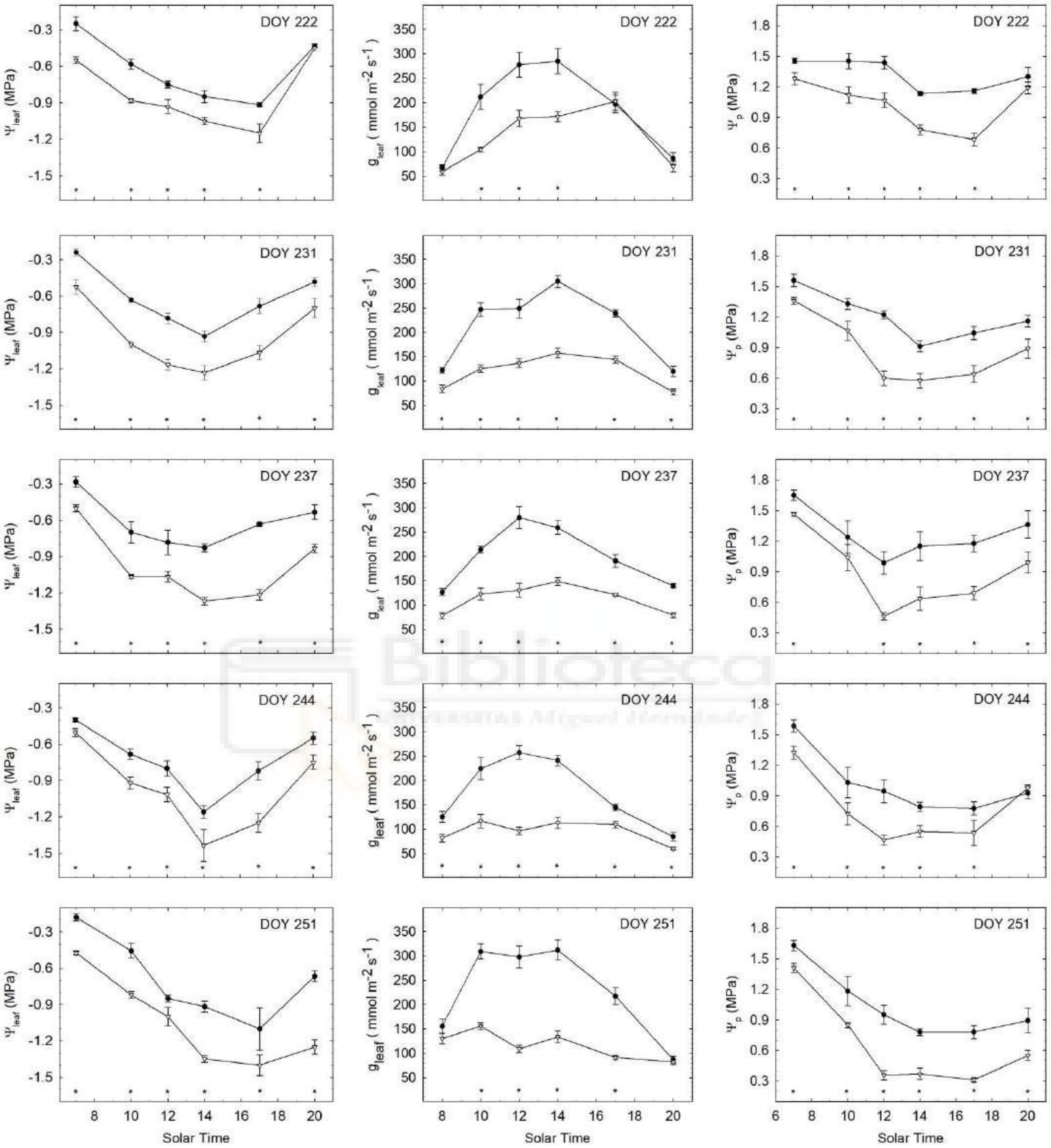

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Fig. 4. Diurnal course of leaf water potential (Ψ_{leaf}), leaf conductance (g_{leaf}) and leaf turgor potential (Ψ_p) values for persimmon plants in T0 and T1 treatments at five different times during the stress period (DOY 222, 231, 237, 244 and 251). Symbols as in Fig. 1.

In order to study the sensitivity of the measured plant-based water status indicators we considered both continuously and discretely recorded plant-based indicators (SF, MDS, Ψ_{pd}, Ψ_{md}, Ψ_{stem} and, g_{lmd}) during increasing intervals of time from the beginning to the end of the experimental period (Table 1). The SI values increased in all plant-based water stress indicators considered in response to water deficit. However, during the experimental period different behaviours were observed. So, at the beginning of the deficit irrigation period the MDS SI (T1/T0) and Ψ_{pd} SI (T1/T0) increased more sharply than SI of the other indicators. After DOY 222, SF SI (T0/T1) values tended to be higher than the SI values of other indicators. Nevertheless, from DOY 218–226 and 218–231, Ψ_{pd} SI (T1/T0) values were similar to those observed in the SF SI. When the mean SI values were considered in relation to their noise for all the plant-based water stress indicators (Table 1), the described behaviours changed. The data indicated that Ψ_{md} mean noise was very low, leading it to show the

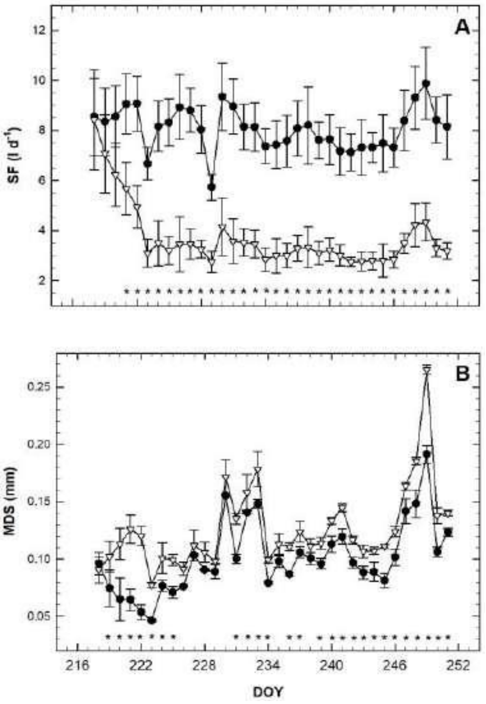

Fig. 5. Daily sap flow (SF) (A) and maximum daily trunk diameter shrinkage (MDS) (B) in T0 and T1 plants during the experimental period. Symbols as in Fig. 1.

highest Ψ_{md} signal:noise ratio at the beginning of the experimentalperiod (DOY 218–222 and 218–226). However, from DOY 226 to the end of the experiment, the substantial increase in the SF SI led to a higher signal:noise ratio for all the following intervals of time considered, even though Ψ_{md} signal:noise ratios were close to those of the SF signal:noise ratio.

At the end of the experimental period (DOY 251), no significant differences in Ψ_{os}, Ψ_{tlp}, Є, RWC_{tlp} or RWC_a values were found between T0 and T1 plants (Table 2). Nevertheless, it is important to point out that RWC_a values were very high in both treatments.

4. Discussion

Throughout the experimental period and based on the fact that (i) θ_v values in T0 treatment were slightly above field capacity (Fig.1), (ii) Ψ_{pd}, Ψ_{md} and Ψ_{stem} values (Figs. 2A, 2 B and 3 A) were nearly constant and very high in relation to the values already reported for other authors for full irrigated persimmon plants (Badal et al., 2010; Buesa et al., 2013), and (iii) Ψ_{pd} values depend mainly on soil moisture levels (Elfving et al., 1972; Torrecillas et al., 1988; Sellin, 1996), we conclude that T0 plants were under non-limiting soil water conditions. Moreover, considering that the tree water relations under flooding conditions are characterized by a substantial decrease in leaf conductance and leaf water potential as a consequence of the effects

Table 1
Mean signal intensity (actual value/reference value or reference value/actual value), mean noise (coefficient of variation), and signal:noise ratio of máximum daily trunk shrinkage (MDS), sap flow (SF), predawn (Ψ_{pd}), midday stem (Ψ_{stem}) and midday (Ψ_{md}) water potentials and midday leaf conductance (g_{lmd}) at different intervals of the experimental period. For each interval, mean signal or mean noise values that do not have a common letter are significantly different according to Duncan's multiple range test (P ≤ 0.05).

DOY		Mean signal	Mean noise	Signal:noise
218-222	MDS	1.65a	0.31bc	5.33
	SF	1.40bc	0.24cd	5.95
	Ψ_{pd}	1.60ab	0.53a	3.01
	Ψ_{stem}	1.20cd	0.24cd	5.03
	Ψ_{md}	1.12d	0.15d	7.37
	g_{lmd}	1.33cd	0.35b	3.76
218-226	MDS	1.54c	0.26b	5.82
	SF	1.85a	0.32ab	5.71
	Ψ_{pd}	1.78ab	0.40a	4.50
	Ψ_{stem}	1.37cd	0.26b	5.30
	Ψ_{md}	1.16d	0.12c	9.88
	g_{lmd}	1.56bc	0.34ab	4.57
218-231	MDS	1.40bc	0.27abc	5.24
	SF	2.04a	0.27abc	7.56
	Ψ_{pd}	1.88a	0.33a	5.62
	Ψ_{stem}	1.43bc	0.22bc	6.46
	Ψ_{md}	1.24c	0.18c	7.00
	g_{lmd}	1.63b	0.29ab	5.62
218-237	MDS	1.34c	0.24abc	5.49
	SF	2.16a	0.23abc	9.40
	Ψ_{pd}	1.86b	0.30a	6.28
	Ψ_{stem}	1.42c	0.19abc	7.40
	Ψ_{md}	1.27c	0.16c	8.05
	g_{lmd}	1.73b	0.28ab	6.26
218-244	MDS	1.30c	0.22ab	5.85
	SF	2.25a	0.20ab	11.13
	Ψ_{pd}	1.94b	0.28a	6.92
	Ψ_{stem}	1.47c	0.20ab	7.24
	Ψ_{md}	1.27c	0.15b	8.65
	g_{lmd}	1.89b	0.30a	6.18
218-251	MDS	1.29c	0.20bc	6.37
	SF	2.29a	0.18bc	12.55
	Ψ_{pd}	2.03b	0.28ab	7.36
	Ψ_{stem}	1.48c	0.19bc	7.96
	Ψ_{md}	1.25c	0.14c	8.84
	g_{lmd}	2.01b	0.32a	6.35

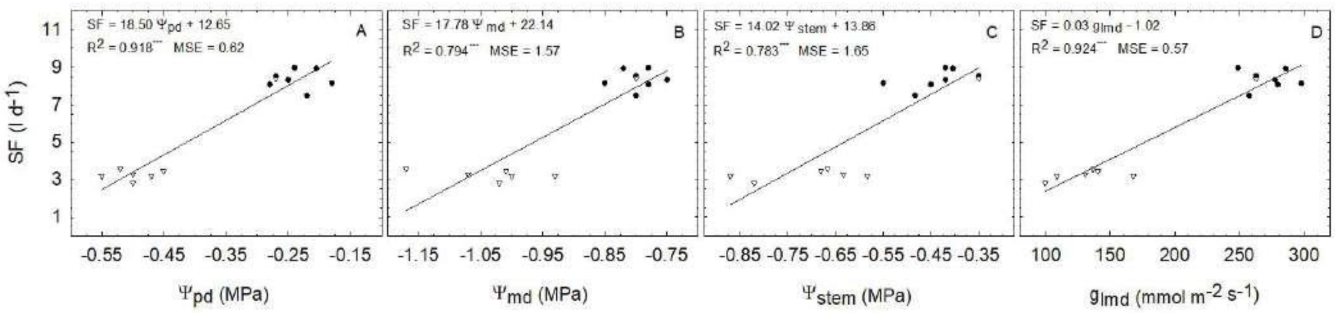

Fig. 6. Relationships between sap flow (SF) and predawn leaf water potential (Ψ_{pd}, A), midday leaf water potential (Ψ_{md}, B), midday stem water potential (Ψ_{stem}, C) and midday leaf conductance (g_{lmd}, D) values for persimmon plants in T0 and T1 treatments during the measurement period. Symbols as in Fig. 1.

Table 2
Effect of water stress on leaf osmotic potential at full turgor (Ψ_{os}), leaf osmotic potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ), relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) of persimmon plants in T0 and T1 treatments at the end of the experimental period. Means with the same letter across each row do not differ significantly at $P \leq 0.05$ (n = 4).

Parameters	T0	T1
Ψ_{os} (MPa)	−1.41a	−1.50a
Ψ_{tlp} (MPa)	−2.76a	−3.32a
ϵ (MPa)	2.50a	3.38a
RWC_{tlp} (%)	77.50a	76.95a
RWC_a (%)	58.09a	56.94a

of chemical signals from roots and an increase in the resistance to water flowing through the plant (Ruiz-Sánchez et al., 1997; Dell'Amico et al., 2001), the water relations of T0 plants indicated the absence of any waterlogging because leaf turgor was maintained (Ψ_{ppd} and $\Psi_{pmd} > 0$), and high and near constant values of Ψ_{pd}, Ψ_{md}, Ψ_{stem}, SF and g_{lmd} were observed (Figs. 2A, B, 3 A, B and 5 A). Regarding the T1 treatment, the fact that minimum θ_v values were around 81% of field capacity and minimum Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} values were around - 0.50 MPa, - 1.17 MPa, - 0.87 MPa and 99.66 mmol m^{-2} s^{-1}, respectively, indicated that T1 plants were under a mild degree of water deficit during the experimental period (Figs. 2A, 2 B, 3 A and 3 (Cruz et al., 2012; Rodríguez et al., 2012; Torrecillas et al., 2018). In addition, the rate of development of water stress in T1 plants was very low because the Ψ_{pd}, Ψ_{stem} and Ψ_{md} values decreased by only around 0.01, 0.02 and 0.01 MPa per day basis (2A, 3A and 2B, respectively) (Hale and Orcutt, 1987).

The progressive decrease in g_{lmd} in T1 plants, and the tendency to shorten the duration of maximum stomatal opening in its circadian rhythm as stress progressed (Figs. 3B and 4) indicated that stomata regulation is a key mechanism in controlling leaf water status because leaf turgor was maintained in T1 plants (Fig. 2C and D) and persimmon plants did not develop any other stress tolerance mechanism such as elastic adjustment (ϵ decrease) or active osmotic adjustment (Ψ_{os} decrease) in our experimental conditions (Table 2). The decrease in g_{lmd} values of woody crop leaves in response to water deficit has been reported as a stress avoidance mechanism in response to water deficit, which improves water use efficiency (Rieger and Duemmel, 1992; Girona et al., 1993). The behaviour of ϵ, Ψ_{os}, Ψ_{tlp} and RWC_{tlp} values, which did not change as a result of water deficit in T1 plants (Table 2), was similar to the results obtained by other authors (Sánchez-Blanco et al., 1991; Savé et al., 1995; Torrecillas et al., 1996) suggesting that the ϵ and Ψ_{os} affect the RWC_{tlp} and Ψ_{tlp} values, respectively. RWCa values in persimmon plants (around 58%) (Table 2) were similar to those found for grapes (51–63%) (Rodrigues et al., 1993), to the lower limit of the range found for *Pinus ponderosa* (57–81 %) (Hardegree, 1989). and to the higher limit found for pomegranate (42–58%) (Rodríguez et al., 2012) and almond (42–59%) (Torrecillas et al., 1996). On the other hand, persimmon RWCa values were high compared with other tree species such as apricot (27–42%) (Torrecillas et al., 1999), peach (29–44%) (Mellisho et al., 2011), *Eucalyptus globulus* (14–27%) (Correia et al., 1989) and *Quercus alba* (26–31%) (Parker and Pallardi, 1987). High RWC_a values represent a xeromorphic characteristic (Cutler et al., 1977), and are a consequence of thicker cell walls or differences in cell wall structure (Hellkvist et al., 1974), which could contribute to the retention of water when water potential decreases (Torrecillas et al., 1996).

SF, Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} encompass different time scales, because the last four are point measurements, taken at predawn or at midday, and are considered as indicators of the minimum (Ψ_{pd}) and maximum (Ψ_{md}, Ψ_{stem} and g_{lmd}) daily plant water deficit, whereas SF is an integrative indicator, which reflects the continuous sap flow records on a diurnal basis. Despite these facts, the relationships between SF and Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} (Fig. 6) were high and constant, indicating that SF can be used to indicate the water status of young persimmon trees. Assuming that any comparison of the sensitivities of different plantbased water status indicators for diagnosing water deficit must consider the strength of each indicator in the context of its variability (Goldhamer and Fereres, 2001; Naor and Cohen, 2003), it can be observed that Ψ_{md} was the most suitable indicator for persimmon irrigation scheduling when short periods of time are considered, because it showed the highest signal:noise ratio during the first 4 or 8 days of the experimental period (DOY 218–222 and 218–226) (Table 1). However, as the interval of time considered grew (DOY 218–231, 218–237, 218–244 and 218–251) SF SI sharply increased and SF noise was maintained, leading it to show the highest signal:noise ratio for these intervals of time (Table 1). Moreover, taking into consideration that during the two first periods of time considered (DOY 218–222 and 218–226) the SF signal:noise ratio, despite being lower than that showed by Ψ_{md} was relatively high, it could be concluded that SF is a more suitable indicator than Ψ_{md} for irrigation scheduling because it can provide continuous and automated registers of the plant water status in real time, avoiding frequent trips to the field and a significant input of manpower since frequent Ψ_{md} readings are needed. In this respect, Ortuño et al. (2004) indicated that in young trees continuously measured plant water status indicators were more immediate and sensitive than discretely measured indicators for detecting water stress. Also, other authors indicated that MDS and SF revealed significant differences between irrigation treatments even in the absence of differences in Ψ_{stem} (Goldhamer et al., 1999; Remorini and Massai, 2003). By contrast, in persimmon plants, Badal et al. (2010) assessed the feasibility of using MDS, Ψ_{stem}, g_{lmd} and fruit diameter variations and concluded that although MDS can be successfully used as continuous plant water stress indicator, Ψ_{stem} was the most sensitive plant water stress indicator. The above results indicated that persimmon plants exposed to mild water stress are able to gradually develop stomata control (a stress avoidance mechanism). Also, under water stress the high relative apoplastic water content could contribute to the retention of water. So, both drought resistance characteristics could have contributed to the leaf turgor maintenance observed during the experimental period. In addition, the discrete and continuously recorded plant-based indicators showed different degrees of sensitivity for diagnosing persimmon tree water status. Overall, SF measurements made by the heat-pulse technique are the most suitable method for estimating persimmon water status, because it showed the highest signal:noise ratio in almost all intervals of time considered, while providing continuous and automated registers of the persimmon water status in real time.

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7.3 Publication 3 (Open Access)

Leaf mechanisms involved in the response of *Cydonia oblonga* trees to water stress and recovery

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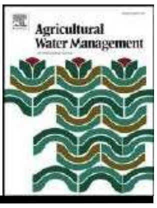

Leaf mechanisms involved in the response of *Cydonia oblonga* trees to water stress and recovery

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ABSTRACT

Quince tree (*Cydonia oblonga* Mill.) is known for bearing fruits that are rich in nutrients and health-promoting compounds while requiring low inputs of agrochemicals, and maintenance, but no information exists on the mechanisms developed at the level of leaf water relations to confront water stress and recovery. For this reason, the purpose of the present study was to identify the strategy (isohydric or anisohydric) by which quince plants cope with water stress and to further elucidate the resistance mechanisms developed in response to water stress and during recovery. In summer 2016, field-grown own rooted 17-years old quince trees (cv. BA-29) were subjected to two irrigation treatments. Control (T0) plants were drip irrigated (105% ETo) to ensure non-limiting soil water conditions, while T1 plants were irrigated at the same level as used in T0, except that irrigation was withheld for 42 days during the linear fruit growth phase, after which irrigation returned to the levels of T0 (recovery period). During the experimental period, T0 and T1 received a total of 374 and 143 mm water, respectively, including rain water. The quince trees exhibited extreme anisohydric behaviour under the experimental conditions. As water stress developed and during the recovery period, the plants exhibited high hydraulic conductivity, probably the result of resistance to cavitation. From the beginning of water stress to the time of maximum water stress, leaf turgor was maintained, possibly due to active osmotic adjustment (stress tolerance mechanism). This leaf turgor maintenance may have contributed to the high leaf conductance, and, therefore, good leaf productivity. The low quince leaf apoplastic water fraction under water stress could be considered as another drought tolerance characteristic because if the accumulation of water in the apoplasm is avoided a steeper gradient in water potential between the leaf and the soil can take place under water stress, thus favouring water absorption.

Abbreviations: Є, leaf bulk modulus of elasticity; ΨL, minimum leaf water potential; Ψleaf, leaf water potential; Ψmd, midday leaf water potential; Ψpd, predawn leaf water potential; Ψpmd, midday leaf turgor potential; Ψppd, predawn leaf turgor potential; Ψos, leaf osmotic potential at full turgor; Ψs, soil water potential; Ψsmd, midday leaf osmotic potential; Ψspd, predawn leaf osmotic potential; Ψstem, midday stem water potential; Ψtlp, leaf water potential at the turgor loss point; gleaf, leaf conductance; glmd, midday leaf conductance; RWC, relative leaf water content; RWCa, relative leaf apoplastic water content; RWCo, relative leaf water content at full turgor; RWCtlp, relative leaf water content at the turgor loss point.

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7.3 Response of *Cydonia Oblonga* to water stress

1. Introduction

Modern agricultural systems have promoted the cultivation of high-input and high-yielding crop species, leading to the intense cultivation of a restricted number of species and a decline in the cultivation of many traditional fruit crops, resulting in a worldwide reduction in crop diversity (Chivenge et al., 2015). However, many of these neglected or underutilized species are not only critical for the diversity of human diets, but can also contribute to increasing food production, providing a more sustainable and resilient agro- and horti-food system (Baldermann et al., 2016). In view of the likelihood that climate change will generate more frequent and severe drought periods, one effective measure to attain sustainable agriculture in arid and semiarid agrosystems might be to introduce underutilized crop species, cultivars and even rootstocks that require low inputs of both agrochemicals and water, while providing attractive fruits that are rich in nutrients and health-promoting compounds.

Quince (*Cydonia oblonga* Mill.) is one example of an underutilized crop species. A shrub or small deciduous tree, quince is a member of the genus *Cydonia* in the family Rosaceae, subfamily Spiraeoideae, tribe Pyreae and subtribe Pyrinae (Postdam, 2012), along with apple (*Malus* sp.) and pear (*Pyrus* sp.). It is thought to originate in the foothills of the trans-Caucasus region including Armenia, Azerbaijan, Iran, south-western Russia, and Turkmenistan. Many of the cultivars described over 100 years ago are still cultivated today (Postdam, 2012). Quince does not require intensive maintenance and can grow in many warm-temperate and temperate regions of the world. It has also become a key factor for other crop cultures, being, for instance, the most important rootstock for pear cultivation (Gur et al., 1978).

Quince fruit is a member of the pome fruit species. Its fruits are climateric, with a pear or apple shape and attractive golden yellow colour. Quince fruits have excessive astringency, sourness and woodiness at harvest, but a pleasant, lasting, and powerful flavour when ripe (Szychowski et al., 2014). In many countries, quince-based products such as jam, jelly, cakes and liquors are much appreciated. The most distinguishing characteristics of quince fruit are its low fat content and very high content of organic acids, sugars, crude fibre, minerals and health-promoting constituents with antioxidant effects (Silva et al., 2004; Fattouch et al., 2007; Shinomiya et al., 2009). Quince fruits are also known for their hypoglycemic, anti-inflammatory, anticarcinogenic, antimicrobial, anti-allergic and antiulcerative properties and the ability to act as a tonic for heart and brain (Hamauzu et al., 2005; Shinomiya et al., 2009; Gur et al., 1978). Despite these many desirable characteristics, consumption of the fresh quince fruit or quince-based products is not widespread.

However, to date, despite the very important advantages to be had as a result of eating quince and the ever more frequent water shortages experienced in the most suitable regions for its growth, the leaf water relations of quince trees in response to drought are unclear. In this sense, Galindo et al. (2018) suggested that fruit trees present different mechanisms to confront water deficit, even though most of them at leaf level resist dehydration through drought avoidance and tolerance mechanisms (Torrecillas et al., 2018). For this, the research reported in this paper was conducted to test the hypothesis that i) quince trees at leaf level resist dehydration through drought avoidance and tolerance mechanisms, and that ii) quince plants are able to improve their water stress resistance by means of an anisohydric strategy.

2. Materials and methods

2.1. *Experimental conditions, plant material and treatments*

Table 1
Soil characteristics of the experimental plot.

Parameters*	
pH	8.37
Electrical conductivity (dS m^{-1})	0.46
Sand (%)	26.2
Loam (%)	37.2
Clay (%)	36.6
Active $CaCO_3$ (%)	13.6
Oxidisable organic C (g kg^{-1})	9.67
Total Kjeldahl N (g kg^{-1})	1.44
Available P (mg kg^{-1})	64
Exchangeable K (g kg^{-1})	0.44
Exchangeable Ca (g kg^{-1})	3.67
Exchangeable Mg (g kg^{-1})	0.65

* Values on a dry matter basis.

The experimental plot was located at the farm of the Miguel Hernández Polytechnic University of Elche, near the city of Orihuela (Spain) (38° 4′N, 0° 59′W). The soil was a clay loam Xerofluvent (Soil Survey Staff, 2006), which showed high active calcium carbonate and low organic matter content, electrical conductivity, available phosphorus and potassium exchange levels (Table 1). The irrigation water had a Cl^{-} concentration of 71–84 mg L^{-1} and an electrical conductivity of between 1.4 and 1.6 dS m^{-1}. The plant material consisted of own rooted 17-year old quince trees (*C. oblonga* Mill.), cv. BA-29, planted at 4 m × 5 m. Pest control and fertilization practices were those typically used by local farmers; no weeds were allowed to develop within the orchard using herbicides. Air temperature, solar radiation, air relative humidity, wind speed (2 m above the soil surface) and rainfall were recorded every 15 min by an automatic micrometereological station located near the experimental site. Mean daily air vapour pressure deficit (VPDm, kPa) and ETo (mm) were calculated according to Allen et al. (1998).

The experiment had a randomized complete block design, with two treatments and four replications. Control plants (T0) were irrigated daily during the night using a drip irrigation system with one lateral line per tree row and six emitters (each delivering 3 l h^{-1}) per plant to ensure non-limiting soil water conditions (105% ETo). T1 plants were irrigated as T0, except that irrigation was withheld during the linear fruit growth phase (day of the 2016 year (DOY) 209–251), before restoring irrigation at the same level as used in T0 from DOY 251–266 (recovery period). During the experimental period, T0 and T1 received a total of 374 and 143 mm water, respectively, including rain water. Each experimental plot consisting of three adjacent tree rows, each with seven trees very similar in appearance (ground shaded area, height, leaf area, trunk cross sectional area, etc.). The inner plants of the central row of each replicate were used for measurements.

2.2. *Measurements*

Indicators of leaf water relations were measured at midday (12 h solar time) and hourly throughout DOY 224, 238 and 251, using fully expanded leaves from the south-facing side and middle third of the tree. Leaf conductance (g_{leaf}) was measured in two leaves per tree, using a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface. Leaf water potential (Ψ_{leaf}) was measured in two leaves per tree, using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), as recommended by Turner (1988). Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in a similar number and type of leaves as those used for

Ψ_{leaf}, enclosing leaves in a small black plastic bag covered with aluminium foil for at least 2 h before measurements in the pressure chamber (Fulton et al., 2001; Shackel, 2011). Ψ_{leaf}, Ψ_{stem} and g_{leaf} were measured in two leaves per replicate in order to improve the representativeness and accuracy of the data and to decrease statistical bias. The values for each replicate were averaged.

After measuring predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials, the leaves were frozen in liquid nitrogen and the osmotic potentials (Ψ_{spd} and Ψ_{smd}, respectively) were measured after thawing thesamples and expressing sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Predawn (Ψ_{ppd}) and midday (Ψ_{pmd}) leaf turgor potentials were derived as the difference between osmotic and water potentials.

Estimates of predawn leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ), relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) were obtained from pressure-volume (PV) analyses of leaves (Tyree and Hammel, 1972; Tyree and Richter, 1981; Tyree and Richter, 1982. At the end of the stress period, 10 leaves per replicate were sealed in plastic bags immediately after excision and resaturated by dipping their petioles in distilled water for 24 h at 4 °C. The resaturated leaves were weighed using an analytical balance (± 0.1 mg precision), placed in the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber). After being depressurized, the leaf was allowed to transpire outside the pressure chamber on the laboratory bench at room temperature (22 ± 2 °C). Leaves were repeatedly weighed and their balance pressures determined over the full range of the pressure gauge (Kikuta and Richter, 1986). Data for initial saturated weight, intermediate fresh weight (corresponding to values for Ψ_{leaf}), and final dry weight (at 80 °C for 48 h) were used to calculate the relative water content (RWC) (Barrs and Weatherley, 1962).

The curves were drawn using a type II transformation (Tyree and Richter, 1982). When the reciprocal of water potential (Ψ_{leaf}) was plotted against RWC, the resultant relationships displayed both linear and non-linear regions. Extrapolation on the straight portion of the curve obtained for a value of RWC = 1 gave the reciprocal of the Ψ_{os} and extrapolation to the abscissa gave RWC_a. Ψ_{tlp} and RWC_{tlp} were estimated as the intersection between the linear and curvilinear portions of the PV curve. The bulk modulus of elasticity (ϵ) of leaf tissue at 100% RWC (RWC_o) was estimated according to Patakas and Noitsakis (1999) as ϵ (MPa) = (Ψ_{os} - Ψ_{stlp})(100 -RWC_a)/(100 - WC_{tlp}), where Ψ_{stlp} is the osmotic potential at the turgor loss point and Ψ_{os} values correspond to those obtained from the analysis of the PV curves.

The methodology proposed by Martínez-Vilalta et al. (2014) was used to categorize the strategy (isohydric or anisohydric) by means of which quince plants cope with drought stress. The above authors assumed that, within biologically reasonable ranges of water potentials, the relationship between soil water potential (Ψ_s) and minimum leaf water potential (Ψ_L) becomes linear ($\Psi_L \approx \Lambda + \sigma \Psi_s$), assuming that soil and plant water potential equilibrate overnight so that Ψ_{pd} and Ψ_{md} are proxies of Ψ_s and Ψ_L, respectively. The slope (σ) indicates the rate of reduction in Ψ_L as Ψ_s declines. Specifically, (i) when Ψ_L remains constant as Ψ_s diminishes ($\sigma = 0$), plants show a strictly isohydric behaviour, (ii) when the difference between Ψ_L and Ψ_s remains constant (σ = 1), plants show a strictly anisohydric behaviour, (iii) when the difference between Ψ_L and Ψ_s is reduced as Ψ_s diminishes ($0 < \sigma < 1$), plants show a partial isohydric behaviour, and (iv) when the pressure drop increases through the plant as Ψ_s diminishes ($\sigma > 1$), plants show an extremely anisohydric behaviour.

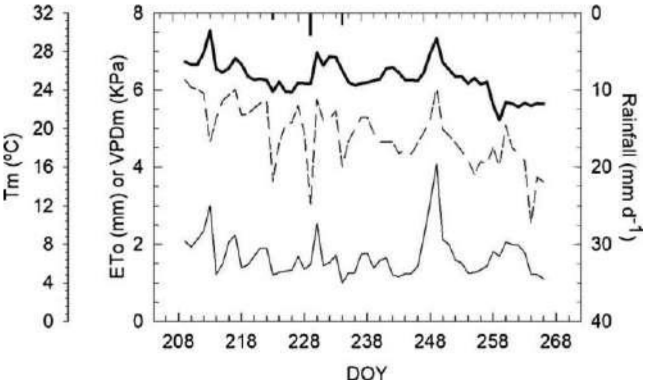

Fig. 1. Daily crop reference evapotranspiration (ETo, medium-medium line), daily mean air temperature (Tm, solid line), mean daily air vapour pressure deficit (VPDm) (thin line) and daily rainfall (vertical bars) during the experimental period.

2.3. *Statistical analysis*

Statistical analysis was performed by an analysis of variance (ANOVA) using the general linear model (GLM) of SPSS v. 12.0 (SPSS Inc., 2002), for which an independent variable (irrigation), having two different levels (T0 and T1), was considered. To check statistical hypothesis (linearity, homoscedasticity, normality and independency) Kolmogorov–Smirnov with the Liliefors correction was used. Shapiro–Wilk and Levene tests were used to evaluate normality and homoscedasticity on the typified residuals, respectively. Independency was assumed by the experimental desing. Regression analysis was made for Ψ_{pd} and Ψ_{md} values with average data of each treatment by date, and statistical hypothesis was checked as for ANOVA.

3. Results

The experimental period (DOY 209–266) was characterized by a VPDm ranging from 0.99 to 4.06 kPa, 282 mm of ETo, 5 mm of rainfall, which occurred mainly on DOY 229 (4 mm) (Fig. 1) and average daily maximum and minimum air temperatures of 32 and 19 °C respectively (data not shown). In other words, the climate of the area was typically Mediterranean, hot and dry during the summer with very scarce rainfall.

During the water stress period, the Ψ_{pd} values showed significant differences between treatments, the Ψ_{pd} values in T0 plants being significantly higher than those in T1 plants (Fig. 2A). The Ψ_{pd} values of the T0 plants were high and showed minimal fluctuations during the experimental period, with average values of -0.48 MPa, whereas the Ψ_{pd} values in T1 plants showed a tendency to decrease during the water withholding period, reaching minimum values at the end of this period (−1.27 MPa) and showing similar values to those of T0 plants at the end of the recovery period (Fig. 2A).

Ψ_{md} values in T0 and T1 plants tended to decrease during the water stress period, even though Ψ_{md} values in T0 plants were higher than those in T1 plants, except on DOY 217 and 238 (Fig. 2B). At the end of the water stress period, plants from both treatments had very low Ψ_{md} values of −3.15 and −3.75 MPa, respectively. When irrigation in T1 plants resumed, Ψ_{md} values recovered to reach similar values to those observed in T0 plants (Fig. 2B).

Ψ_{ppd} and Ψ_{pmd} values in T0 and T1 plants were always above zero, which indicates how turgor was maintained throughout the

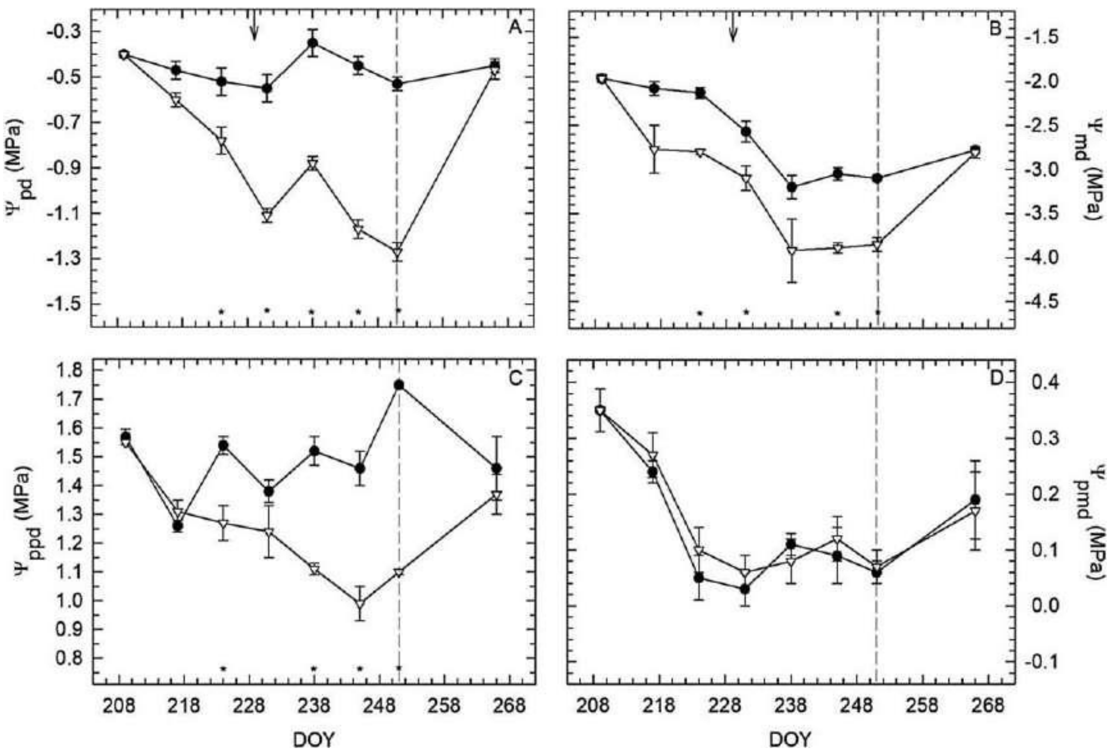

Fig. 2. Predawn (Ψ_{pd}, A) and midday (Ψ_{md} B) leaf water potential, and predawn (Ψ_{ppd}, C) and midday (Ψ_{pmd}, D) leaf turgor potential values (mean ± SE, not shown when smaller than symbols, n = 4) for quince plants in T0 (closed circles) and T1 (open triangles) treatments during the experimental period. Asterisks indicate significant differences between treatments at $P = 0.001$ (*). Vertical dashed line indicates the end of the water stress period. Arrows in A and B indicate daily rainfall events.

experimental period (Fig. 2C and D). Ψ_{ppd} values in T0 plants fluctuated moderately, showing average values of 1.49 MPa, whereas Ψ_{ppd} values in T1 plants decreased to reach values of 1.10 MPa at theend of the irrigation water withholding period but recovered when irrigation resumed. No differences between treatments were found in Ψ_{pmd} values, which fell in both plant treatments during the measurement period, reaching values of only 0.07 MPa and increasing slightly when irrigation was restarted in T1 plants.

Ψ_{stem} values in T0 and T1 plants showed a qualitative behaviour very similar to that shown by Ψ_{md} values during the experimental period, even though Ψ_{stem} values in both irrigation treatments were higher than the corresponding Ψ_{md} values Figs. 2B and 3 A). The Ψ_{stem} values of T1 plants had decreased by DOY 217, remaining lower than the corresponding values in T0 plants from that date onwards, except on DOY 224 and 231, reaching values of -2.35 MPa at the end of the stress period and recovering when irrigation resumed (Fig. 3A).

Midday leaf conductance (g_{lmd}) values in T0 plants were high and fairly constant throughout the experimental period, showing average values of 323 mmol m^{-2} s^{-1} (Fig. 3B). In contrast, water stress induced a gradual reduction in g_{lmd} values, before recovering when irrigation resumed. This reduction during the stress period can be considered as moderate because average g_{lmd} values in T1 plants decreased by 31%, although at the end of water withholding period g_{lmd} values of 178 mmol m^{-2} s^{-1} were recorded (Fig. 3B).

During the water withholding period, Ψ_{leaf} values in T0 and T1 plants on DOY 224, 238 and 251 showed significant differences between treatments but a very similar daily time course, which was characterized by a gradual decrease in Ψ_{leaf} values during the early morning, reaching minimum values at around (13:00 - 15:00 h) and recovering in the afternoon (Fig. 4). On DOY 224, 238 and 251, these minimum Ψ_{leaf} alues in T0 plants were −3.03, −3.20 and −3.15

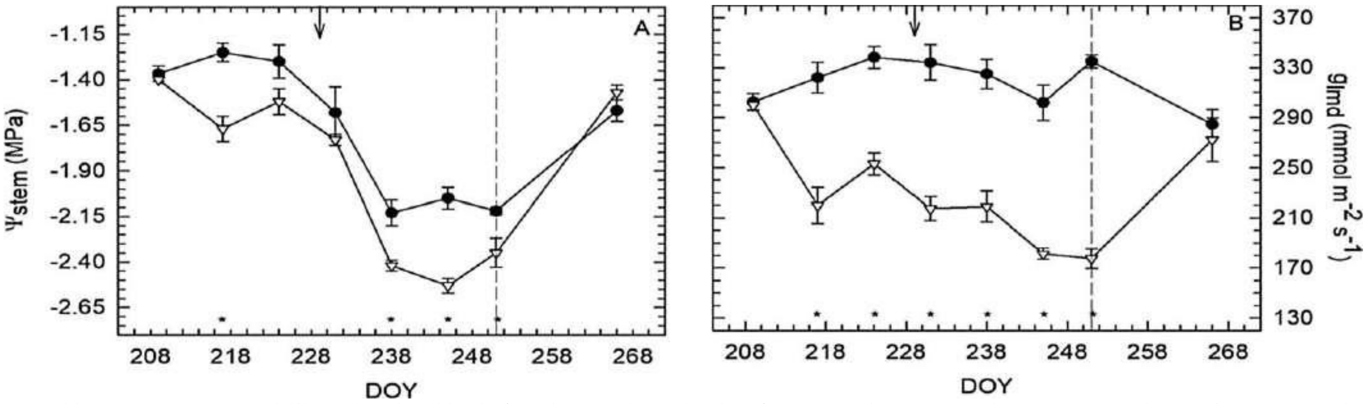

Fig. 3. Midday stem water potential (Ψ_{stem}, A) and midday leaf conductance (g_{lmd}, B) values for quince plants in T0 and T1 treatments during the experimental period. Symbols as in Fig. 2

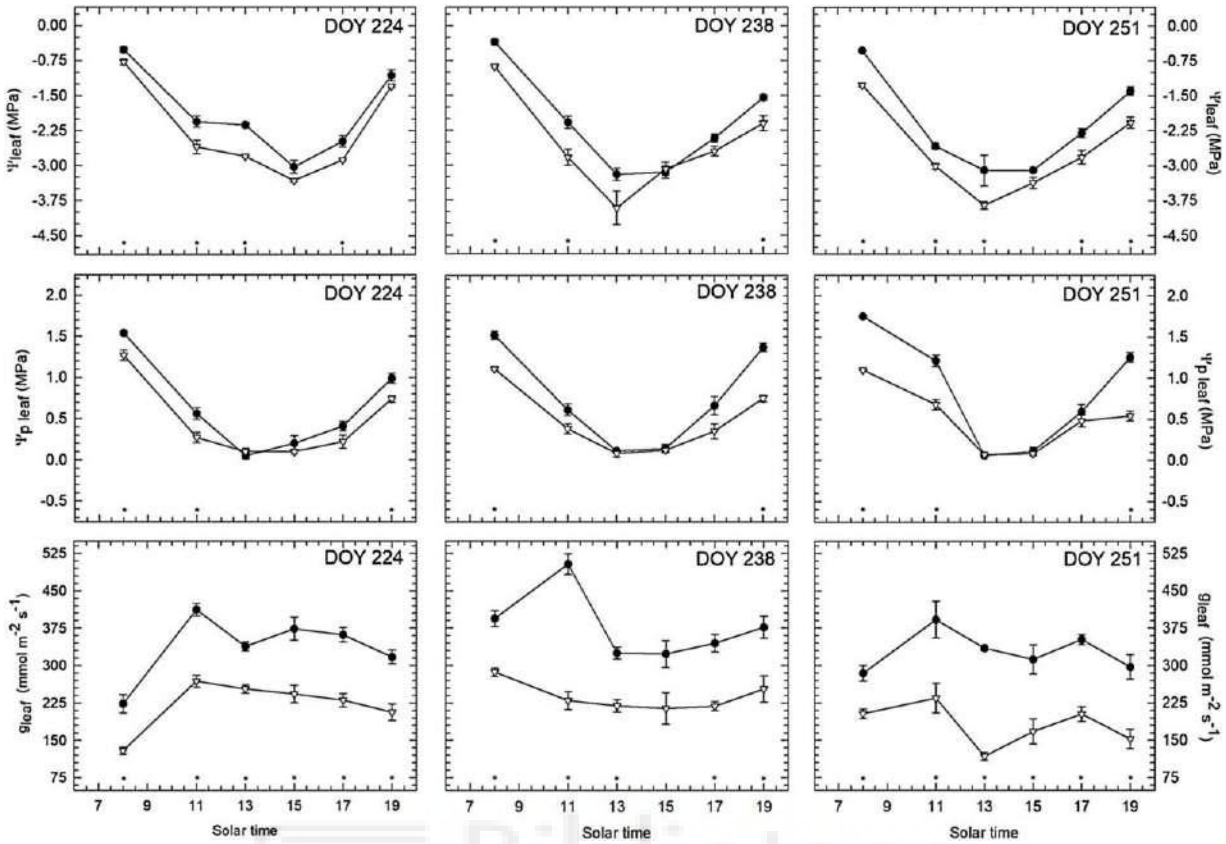

Fig. 4. Diurnal course of leaf water potential (Ψ_{leaf}), turgor potential ($\Psi_{p\ leaf}$) and leaf conductance (g_{leaf}) values for quince plants in T0 and T1 treatments at three different times during the stress period (DOY 224, 238, 251). Symbols as in Fig. 2

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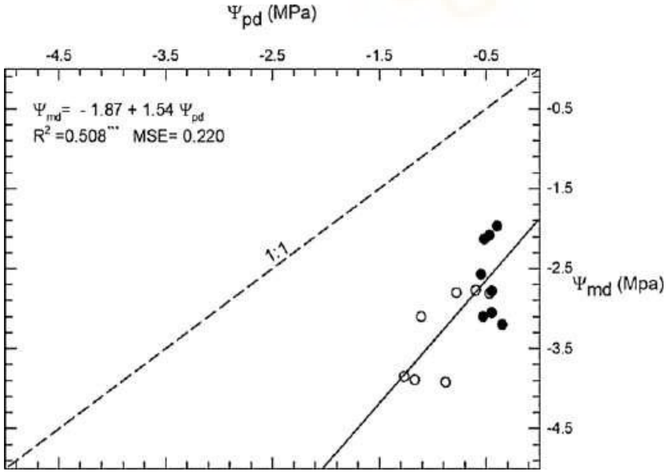

Fig. 5. Relationship between predawn (Ψpd), and midday (Ψmd) leaf water potentials, for quince plants in T0 (closed circles) and T1 (open circles), according to the theoretical model of Martínez-Vilalta et al. (2014), which assumes a linear relationship with four different behaviours, all sharing the same intercept: strictly isohydric (σ (slope)=0), partially isohydric (0 < σ < 1), strictly anisohydric (σ=1) and extremely anisohydric (σ < 1). Each value is the mean of four replicates.

MPa, respectively, and in T1 plants they were −3.32, −3.92 and −3.75 MPa, respectively. Differences between treatments in leaf turgor values ($\Psi_{p\ leaf}$) were lower than those observed for Ψ_{leaf} values, and occurred mainly during the early morning and late afternoon (Fig. 4). $\Psi_{p\ leaf}$ values presented a diurnal time course very similar to that exhibited by Ψ_{leaf} values, even though daily minimum $\Psi_{p\ leaf}$ values, which stabilized from 13:00 to 15:00 h, were very low and ranged between 0.05 and 0.11 MPa (Fig. 4).

The circadian rhythm of g_{leaf} values in T0 plants was characterized by an increase around sunrise because of the stomata opening, a peak at around 10:00 h, followed by a slight decreased and stable values until late afternoon. In response to the withholding of irrigation water, g_{leaf} values in T1 plants showed low diurnal time course changes, these values being significantly lower than those observed in T0 plants (Fig. 4).

The relation between Ψ_{pd} and Ψ_{md} (Fig. 5), which is used to define the ability of stomata to regulate the leaf water potential, or, in other words, the isohydric/anisohydric behaviour, showed a slope (σ) higher than unity. For this reason, under the studied experimental conditions, quince plants can be said to have exhibited an extreme anisohydric behaviour in response to drought stress.

On DOY 251, the last day of the irrigation water withholding period, Ψ_{os} values were lower in T1 than in T0 plants. However, no significant differences in Ψ_{tlp}, Є, RWC_{tlp} or RWC_a values were found between treatments (Table 2). In addition, it should be noted that Ψ_{tlp} values in both treatments were very low (Table 2).

4. Discussion

The fact that Ψ_{pd} values, which depend on soil moisture levels (Elfving et al., 1972), in cont rol plants (T0) were high and near constant during the experimental period (Fig. 2A) indicated that the irrigation applied to this treatment was sufficient to avoid any water deficit. In contrast, Ψ_{pd}, Ψ_{md} and Ψ_{stem} values in T1 plants decreased at a relatively low rate (0.02, 0.04 and 0.02 MPa d^{-1}, respectively) (Hale and Orcutt, 1987), achieving severe water stress levels (Figs. 2A, B and 3 A).

Table 2
Effect of water stress on leaf osmotic potential at full turgor (Ψ_{os}), leaf osmotic potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ),relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) of quince plants in T0 and T1 treatments at the end of the water withholding period. Means with different letter across each row differ significantly at $P = 0.05$ (n = 4).

Parameters	T0	T1
Ψ_{os} (MPa)	−1.66b	−1.88a
Ψ_{tlp} (MPa)	−4.76	−4.56
ϵ (MPa)	4.66	4.52
RWC_{tlp} (%)	45.33	44.91
RWC_a (%)	18.18	19.31

Under water stress conditions, the large differences between Ψ_{pd} and Ψ_{md} values in T1 plants (Fig. 2A and B) and the recovery of the low Ψ , Ψ and Ψ values when they were rewatered (Figs. 2A, B and 3 D). While the level of osmotic adjustment contributed to leaf turgor maintenance, it was not sufficient to modify the Ψ_{tlp} values (Table 2). Other authors observed no changes in Ψ_{tlp} values despite the fact that these woody crops developed osmoregulation (Rodríguez et al., 2012; Cruz et al., 2012; Mellisho et al., 2011).

Contrary to the behaviour observed in *Citrus* (Savé et al., 1995) and avocado (Sharon et al., 2001), quince plants were not able to develop elastic adjustment (decrease in ϵ) in T1 plants due to the effect of a water deficit (Table 2). Also, the similar behaviours observed for ϵ and RWC_{tlp} values agree with the results of Savé et al. (1995), who suggested that ϵ controlled RWC_{tlp}. Whatever the case, when plants are rehydrated after a water stress period, the maintenance of or an increase in cell wall rigidity may be necessary to maintain cell tissue integrity in species that show osmotic adjustment (Clifford et al., 1998; Álvarez et al., 2009).

The RWCa values in quince plants of 18–19 % (Table 2), were lower than those found in other fruit trees (Rodrigues et al., 1993; Torrecillas et al., 1996, 1999; Mellisho et al., 2011; Cruz et al., 2012; Rodríguez et al., 2012) as a consequence of thinner cell walls or differences in cel wall structure (Hellkvist et al., 1974). In contrast with observations A) has also been observed in other crops subjected to severe water stress (Torrecillas et al., 1996; Ruiz-Sánchez et al., 1997; Rodríguez et al., 2012 and may be related with high hydraulic conductivity, probably as a result of the resistance to cavitation observed in anisohydric plants (Ewers et al., 2005; Alsina et al., 2007).

In T1 plants, the gradual reduction in g_{lmd} values from the beginning of the stress period (Fig. 3B) can be considered as a primary response to irrigation water withholding, which improves water use efficiency (Rodríguez et al., 2012; Rieger and Duemmel, 1992). The low level of stomatal regulation (Figs. 3B and 4), even when minimum Ψ_s levels (estimated as Ψ_{pd} values) were achieved (Figs. 2A, 3 B and 4), led to pronounced decreases in Ψ_{md} values in T1 plants. The Ψ_{md} values in T0 and T1 plants correlated with Ψ_{pd} values and showed a slope higher than unity ($\sigma = 1.54$) (Fig. 5). For this reason, and in agreement with Martínez-Vilalta et al. (2014), quince plants can be categorized as being extremely anisohydric, which improves the drought resistance of the crop (Sade et al., 2012). In this respect, in addition to the above mentioned advantage of being more resistant to cavitation, which permits anisohydric plants to recover rapidly following exposure to water stress, the low rate of stomatal regulation in such plants facilitates higher rates of leaf gas exchange than in isohydric plants (Franks et al., 2007).

Whatever the case, it is important to underline that, as Klein (2014) and Martínez-Vilalta et al. (2014) indicated, very few plant species conform strictly to the definitions of isohydric or anisohydric plants because plant species are ordered on a continuum, along which individual species can move in response not only of their physiological characteristics but also to those in which they grow and the conditions to which they are exposed at any given moment (Schultz, 2003; Domec and Johnson, 2012; Rogiers et al., 2012). Because of this, some plants can shift from being anisohydric to isohydric, depending on Ψ_s (Domec and Johnson, 2012).

The Ψ_{os} values decrease (0.22 MPa) in T1 quince leaves indicated and active accumulation of osmolytes and, consequently, an active osmotic adjustments (Table 2). Similar behaviours have been observed in other fruit trees such as apple (Wang et al., 1995), apricot (Torrecillas et al., 1999), cherry (Ranney et al., 1991), jujube (Cruz et al., 2012), peach (Steinberg et al., 1989; Arndt et al., 2000) and pomegranate (Rodríguez et al., 2012). In this sense, it is known that osmoregulation takes place mainly when water stress develops gradually over a prolonged period (Arndt et al., 2000), as in our experimental conditions, and it varies depending on the species and cultivar (Torrecillas et al., 1996; Lakso, 1990).

The maintenance of turgor in T1 plants even at maximum water deficit levels (Fig. 2C and D), when g_{lmd} values remained considerable (Figs. 3B and 4), suggested that active osmoregulation contributed to maintaining quince leaf turgor (Ψ_{ppd} and Ψ_{pmd} above zero) (Fig. 2C and made in other woody crops (Cruz et al., 2012), the consistently low leaf apoplastic water fraction in quince exposed to water stress prevents the accumulation of water in the apoplasm, leading to lower leaf water potential values and, as a consequence, a steeper gradient in water potential between the leaf and the soil, thus favouring water absorption.

The above results indicated that under our experimental conditions quince trees were characterized by an extreme anisohydric behaviour. During the development of the severe water stress and recovery periods, the plants suggested high hydraulic conductivity, probably because of the resistance to cavitation. From the beginning of water stress to the time of maximum water stress, leaf turgor was maintained probably due to the contribution of the active osmotic adjustment (stress tolerance mechanism). This leaf turgor maintenance would allow substantial leaf conductance levels and, therefore, good leaf productivity. This constantly low quince leaf apoplastic water fraction under water stress could be considered as another drought tolerance characteristic because, if water is prevented from accumulating in the apoplasm, a steeper gradient in water potential between the leaf and the soil may occur during water stress, thus favouring water absorption.

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7.4 Publication 4 (Transcripción literal)

Volatile composition and sensory and quality attributes of quince (*Cydonia oblonga* Mill.) fruits as affected by water stress

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Volatile composition and sensory and quality attributes of quince (*Cydonia oblonga* Mill.) fruits as affected by water stress

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ABSTRACT

Keywords:
HydroSOStainable fruits
Sensory quality
Underutilized crops
Water deficit

No information exists on the effect of water deficit on quince (*Cydonia oblonga* Mill.) fruit quality characteristics. In this paper, the effect of withholding irrigation water in the middle of the rapid fruit growth period on yield, main fruit physicochemical characteristics, sensory profile, and volatile composition of quince (cv. BA-29) was studied. The linear fruit growth phase was seen to be a critical phenological period for the marketable yield of quince because water deficit decreased the fruit weight, size, and moisture content. Also, this period was clearly critical for the fruit's chemical characteristics. Water deficit induced important changes in an important number of the identified volatile compounds, characterized by decreases on pear, pineapple and apple sensory descriptors and increases on the green-herbaceous sensory descriptors. Moreover, total soluble solids increased and the total polyphenols content and antioxidant activity decreased as a result of water deficit effect, even though the still high levels of total polyphenols, regardless of the fruit water status, suggested that quinces can be used as an important source of natural antioxidants. The fruit sensory analysis indicated that the peel colour intensity of fruits from the water deficit treatment increased and fruit flesh texture improved. Indeed, the main handicaps for its consumption as fresh fruit (corkiness and fibreness) severely decreased and crunchiness increased.

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1. Introduction

The fruit tree industry of the Mediterranean basin is facing several important challenges, which are seriously limiting its profitability. Among them, water is considered the main limiting factor for optimal fruit and vegetable farming, and the availability of this resource is drastically being reduced. In addition, Mediterranean agrosystems are economically restricted to the farming of very few plant species of woody crops (Prescott-Allen and Prescott-Allen, 1990). Most of these are high-yielding species, which contribute to the decline in crop diversity in these agricultural systems (Chivenge et al., 2015), which is fundamental for ecosystem functioning, sustainable agricultural production, and increased food and nutritional security (Thrupp, 2000; Toledo and Burlingame, 2006; Chappell and LaValle, 2011). It is important to face up to the fact that climate change will in evitably lead to very frequent and severe droughts in the near future, so that modern sustainable agriculture in arid and semiarid agrosystems will have to cope with water scarcity by adopting deficit-irrigation strategies. This type of irrigation will help to minimize the impact of water shortage on crop yield and quality and even enable the diversification of fruit tree culture turning growers´ attention to a variety of underused fruit tree crops, which may need low inputs of water, synthetic fertilisers and pesticides. Besides, they yield attractive fruits, rich in nutrients and health-promoting compounds, and so have a huge potential for improving nutrition and providing preventive effects against some chronic diseases (Baldermann et al., 2016; Galindo et al., 2018; Rodríguez et al., 2018). These type of vegetables and fruits, grown with deficit-irrigation strategies have been aptly called *hydroSOStainables* (Noguera-Artiaga et al., 2016; Cano-Lamadrid et al., 2017).

To date, there has been no scientific study on the response of quince (*Cydonia oblonga* Mill.) trees to water deficit, even though it has the reputation of being a highly drought-tolerant underappreciated fruit tree that does not require intensive maintenance and can to be grown in many warm-temperate and temperate regions of the world. It is a deciduous tree and the sole member of the genus *Cydonia* in the family Rosaceae, which also contains apples (*Malus* sp.) and pears (*Pyrus* sp.). Quince is a native of the foothills of the Caucasus Mountains between Persia and Turkmenistan, where it has been cultivated for thousands of years for its fruit, and in the rest of the world for hundreds of years as a dwarfing rootstock for pears. The consumption of the fresh quince fruit (quinces) is not widespread mainly due to their excessive and aggressive astringency and sourness and also their woodiness. However, the ripe fruit of selected quince cultivars have a pleasant, lasting, and powerful flavour (Szychowski et al., 2014). Nowadays, the most popular quince-based products are marmalade, jam, jelly, cakes, and liquors, these products that are much appreciated in many countries (e.g. a jam called "*marmalada*" in Portugal, a jelly called "*dulce de membrillo*" in Spain, and "*pigwa*" liquor in Poland) (Silva et al., 2005; Carbonell-Barrachina et al., 2015).

The beneficial health properties of quinces have been known since ancient times, although recent years have seen an increase in attention because of the scientific studies confirming most of these health benefits. For instance, their high antioxidant capacity and interesting phenolic composition have been linked to their hypoglycemic, anti-inflammatory, anticarcinogenic, antimicrobial, antiallergic, and antiulcerative activities (Sharma et al., 2011; Legua et al., 2013; Wojdyło et al., 2013).

Pome fruit growth exhibit a single-sigmoid pattern, with only one period of rapid growth, which is considered a critical phenological period because any water deficit at this time causes a delay in the fruit reaching a marketable fruit size (Domingo et al., 1996; Rodríguez et al., 2018). Indeed, under Mediterranean conditions, the quince rapid fruit growth period coincides with very high values of atmospheric evaporative demand and when water prices increase; all the problems associated with optimizing the effectiveness of the irrigation water available may discourage farmers and ultimately cause land abandonment (Katerji et al., 2008; García-Tejero et al., 2014).

Considering all the above, the aim of the current study was to apply water stress in quince farming, withholding irrigation water in the middle of the rapid fruit growth period and studying the effects of this strategy on yield, the main physicochemical characteristics, sensory profile and volatile composition of quince.

2. Materials and methods

2.1. *Experimental conditions, plant material and treatments*

The experiment was performed during the summer 2016 at an orchard located at the experimental field station of the *Universidad Miguel Hernández de Elche* (*Escuela Politécnica Superior de Orihuela*), near the city of Orihuela (Spain) (38° 4′N, 0° 59′W). The plant material consisted of own rooted adult quince trees (*C. oblonga* (Mill.)), cv. BA-29, spaced following a 4 m × 5 m pattern.

The soil is a Xerofluvent (Soil Survey Staff, 2006) with a clay loam texture. Analytical data showed a high lime content (13.6% active calcium carbonate), very low organic matter content (0.89%), low cationic exchange capacity (6.98 mequiv. 100 g^{-1}), and low available potassium and phosphorus levels. The irrigation water had an electrical conductivity of between limiting soil water conditions (105% ETo during the experimental period). T1 plants were irrigated as T0, except during the middle of linear fruit growth phase (day of the year, DOY, 209–251), when irrigation was withheld. To ensure the recovery of T1 plants, reirrigation was performed at the levels used in T0 from DOY 251 to 291 (end of harvesting period). During the measurements period, total water amounts applied were 374 and 143 mm for T0 and T1 treatments, respectively. Actual precipitation was considered as part of applied water in T0 and T1 plants.

2.2. *Plant water status, fruit weight, size, firmness and colour*

Predawn leaf water potential (Ψ_{pd}) was measured in mature leaves located on the south facing side, from the middle third of the tree (two leaves per tree and four trees per treatment), using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), as recommended by Turner (1988).

Quince fruits were harvested on the 17th of October 2016 (DOY 291). The mean weight of marketable fruit was determined according to the weight and number of fruits per box in randomly selected boxes per replicate. Measurements of the polar and equatorial diameters of the fruit were made in 20 fruits per replicate, using a digital calliper. Flesh firmness (FF) was determined as the load needed to break the flesh after removal of the peel on two opposite faces in the equatorial zone, using a penetrometer (model FT-327, Facchini, Alfonsine, Italy), equipped with an 8 mm cylindrical plunger. For the moisture content, samples were oven-dried at 105 °C until constant weight (AOAC et al., 2000).

Quince peel colour (4 different measurements at 4 equidistant points on the equatorial region of each fruit) was assessed using a Minolta CR 2000 colourimeter (Osaka, Japan) and the results were expressed in the CIE*Lab* system. The mean values for lightness (L^*), red- greenness (a^*), and blue-yellowness (b^*) coordinates for each fruit were reported. In addition, the objective colour was calculated as chromaticity or chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$H^o = \arctan (b^*/a^*)$].

2.3. *Fruit total soluble solids, acidity, maturity index, total phenolics and total antioxidant activity*

Total soluble solids (TSS) and total acidity (TA) were measured in juice samples according to Rodríguez-Guisado et al., 2009, and the maturity index (MI) was calculated as the ratio between TSS and TA.

Freeze-dried quince extract was used to determine the total phenolic compound (TPC) contents following the Cano-Lamadrid et al., 2016 procedure. Quantification was made with respect to a standard curve of gallic acid and the results are expressed as gallic acid equivalents (GAE), mg 100 g^{-1} dw.

The antioxidant activity (AA) was evaluated with three different methodologies ($ABTS^{•+}$, FRAP and $DPPH^{•}$). The $ABTS^{•+}$ (2,2-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation and ferric-reducing antioxidant power (FRAP) methods were applied according to Re et al. (1999), and Benzie and Strain (1996), respectively. The radical scavenging activity was evaluated using the DPPH• radical 2,2-diphenyl1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995) with a modification in the reaction time (Nuncio-Jáuregui et al.,2015).

2.4. *Fruit sensory analysis*

Seven trained panellists from the Food Quality and Safety Group of the Universidad Miguel Hernández de Elche, UMH (Alicante, Spain) participated in this study. Each of the panellists had more than 750 h of testing experience with a variety of food products. For the current study, the panellists received further orientation on fresh and processed quinces.

The evaluation was carried out at the UMH facilities using individual booths with a combination of natural and non-natural (fluorescent) light (70–90 fc) and a temperature of 20 ± 2 °C during three different sessions. Samples were evaluated in triplicate and the sampling order for each panellist was randomized.

The quince samples (3 slices of fresh quince fruit), were served in odour-free, disposable 100 mL covered plastic cups. Three slices of quince fruit (approximately 40–50 g; each slice from a different fruit) were served to each panellist; additional samples were available if the panellists requested it. All samples were served at room temperature. Unsalted crackers and distilled water were used to clean the palate between samples.

Three sessions of 2 h were held for the sample evaluation, and fruits from both treatments (T0 and T1) were assayed in each session. Quince samples were assessed using descriptive sensory analysis. Each panellist had a questionnaire to evaluate the following attributes: colour (peel colour and colour homogeneity), odour (quince, fruity, pineapple, apple and pear), flavour (sourness, sweetness, bitterness, and astringency), aroma (fruity, quince, pineapple, pear, and aftertaste) and texture (hardness, crunchiness, juiciness, graininess and fibreness). The panel developed its own sensory lexicon to describe the quality of quince fruits. The attributes and definitions used for the present study are shown in Table 1. A numerical scale, from 0 to 10 with increments of 0.5, was used, where 0 represents no intensity and 10 represents extremely strong intensity. Results are reported as the mean value ± standard error.

2.5. *Volatile aroma compounds analysis*

Volatile compounds were extracted from quince samples using headspace solid phase micro-extraction (HS-SPME). After several preliminary tests to optimize the extraction system, 5 g of milled quince plus 10 mL of distilled water were placed into a 50 mL vial hermetically sealed with a polypropylene cap and a polytetrafluoroethylene (PTFE)/ silicone septum. One microliter of the internal standard (nonanal) was added together with NaCl and the vial was equilibrated for 15 min at 40 °C in a water bath. Then a 50/30 μm DVB/CAR/PDMS (divinylbenzene / carboxen / polydimethylsiloxane) fibre was exposed to the sample headspace for 50 min at 40 °C.

Isolation and identification of the volatile compounds were performed using a Shimadzu GC-

Table 1
Odour, aroma, flavour, mouthfeel, and texture attributes and definitions used in the study.

[a] o-a stand for odour and aroma, perception of volatile compounds with the food outside or inside the mouth, respectively.

Attribute	Definition	Reference (flavour)
Peel colour	Colour of the quince fruit peel	Pantone 584 U = 2.0 Pantone 604 U = 8.0
Colour homogeneity	Homogeneity of colour in the quince fruit peel	50 % colour homogeneity = 5.0 80 % colour homogeneity = 8.0
Quince ID (o-a)[a]	A floral, fresh, and fruity aromatics associated to quince	Freshly harvested quinces, cv. Vranja = 6.5
Fruity (o-a)	Fresh, fruity aromatics associated with undefined fruits	Hacendado yogurt "Macedonia" = 8.5
Pineapple (o-a)	Sour, fresh, fruity aromatics associated with ripe pineapple	Hacendado 100 % pineapple juice = 7.5
Apple (o)	Sweet, light, fruity, somewhat floral aromatic commonly associated with apple juice and apples	Hacendado mango-apple nectar = 5.5
Pear (o-a)	Sweet, slightly musty, floral, honey/caramel-like, fruity aromatic associated with ripe pears	Hacendado pear nectar = 6.5
Sourness	Fundamental taste factor of which citric acid is typical	0.05 % Citric acid solution = 3.5 0.08 % Citric acid solution = 5.0
Sweetness	Fundamental taste factor of which sucrose is typical	2% Sucrose solution = 2.0 4% Sucrose solution = 4.0
Bitterness	Fundamental taste factor of which caffeine or quinine is typical	0.020% Caffeine solution = 3.5 0.035% Caffeine solution = 5.0
Astringency	Dry puckering mouthfeel associated with an alum solution	0.05 % Alum solution = 2.5
Hardness	Force required to bite through	Fresh unpeeled carrot = 8.5
Crunchiness	The intensity of audible noise at first chew with molars	Fresh unpeeled carrot = 7.5
Juiciness	Amount of water released from the sample during chewing	Raw peeled carrot = 1.5
Corkiness	Puffiness of the cross section	Marshmallow = 1.0
Graininess	Amount of woody particles remaining in the mouth just before swallowing the sample	Over-ripe pear, cv. Alejandrina = 6.0
Fibreness	Stringy particles (size and amount)	Fresh strawberry = 3.0

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17 A gas chromatograph coupled to a Shimadzu QP-5050 A mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The GC/MS system was equipped with a TRACSIL Meta. × 5 column (95% dimethylpolysiloxane / 5 % diphenylpolysiloxane, 60 m × 0.25 mm, 0.25 μm film thickness; Teknokroma S. Coop. C.Ltd, Barcelona, Spain).

The quantification (mg kg^{-1} dry weight, dw) of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC–MS analysis. The injector temperature was 200 °C and nitrogen was used as carrier gas (1 mL min^{-1}). The relative abundance was obtained from electronic integration measurements using flame ionization detection (FID). Nonanal was used as internal standard and the areas from all compounds were normalized using its area; this compound was chosen after checking that it was not present in the volatile profiles of the samples under study

2.6. Statistical design and analysis

The design of the experiment was completely randomized with four replications, each replication consisting of three adjacent tree rows, each with five trees. Measurements were taken on the inner tree of the central row of each replicate, which were very similar in appearance, while the other trees served as border trees. Data were analysed using SPSS software (SPPS, 2002). Analysis of variance was performed and mean values were compared at $p < 0.05$. Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

3.1. Climate, plant water status, yield and fruit physical characteristics

During the experimental period, the climate was hot and dry [average daily maximum and minimum air temperatures were 31 and 19 °C, respectively; VPDm ranged from 0.46 to 4.06 kPa; ETo amounted 357 mm; and, rainfall was 15 mm, mainly on DOY 229 and 287, 10.3 mm]. In other words, the climatic characteristics were typically Mediterranean (Fig. 1).

Ψ_{pd} values for T0 plants were high and almost constant during the experimental period, showing average values of around –0.48 MPa. In contrast, Ψ_{pd} values for T1 plants gradually decreased from the beginning of the water withholding period, reaching minimum values at the end of the stress period (−1.27 MPa) and recovering to T0 levels when irrigation was resumed (Fig. 2).

The applied water stress decreased fruit weight, size, and moisture content and, as a consequence, the yield of marketable quinces declined (Table 2). However, quince fruits under water stress (T1) showed similar fruit firmness to fruits from fully irrigated tress (T0). A significant effect of water stress on some quince peel colour characteristics was observed (Table 3). In this sense, peel from T1 fruits showed higher L* and a* values and lower H°

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Table 5

Effect of irrigation treatments on flesh quince total polyphenols content (TPC, mg GAE 100 g^{-1} dw) and total antioxidant activity (AA, mmol Trolox kg^{-1} dw) measured according to ABTS$^+$, DPPH$^•$ and FRAP assays. Values are the mean of 4 replications. Means within a column that do not have a common letter are significantly different at $p \leq 0.05$.

Treatment	TPC	AA-ABTS$^+$	AA-DPPH$^•$	AA-FRAP
T0	1852.50a	46.40a	62.64a	317.18a
T1	1707.39b	44.56b	60.65a	252.75b

increasing and the last decreasing (Fig. 4). The most abundant compounds (above 400 mg kg^{-1} dw) in T0 fruits were ethyl octanoate (3366 mg kg^{-1} dw), E,E-α-farnesene (2853 mg kg^{-1} dw), and ethyl hexanoate (764 mg kg^{-1} dw), while in T1 fruits the predominant compounds were ethyl octanoate (1739 mg kg^{-1} dw), E,E-α- farnesene (1076 mg kg^{-1} dw), ethyl hexanoate (756 mg kg^{-1} dw), 1- hexanol (496 mg kg^{-1} dw), and butyl acetate (478 mg kg^{-1} dw) (Table 6).

Although the volatile profile was similar in quince fruits from both irrigation treatments (the same compounds were found in the quinces from both treatments), water deficit induced important changes in the content of 27 identified compounds out of a total of 55 (Table 6). In fact, in T1 fruits the most important observed changes were increases in the contents of butyl acetate, 1-hexanol, ethyl hexanoate and butyl acetate, and decreases in the contents of ethyl octanoate and E,E-α- farnesene.

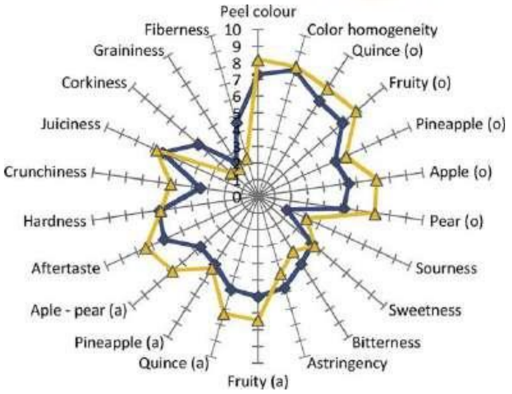

Fig. 3. Descriptive sensory analysis in T0 (blue line) and T1 (yellow line) quince fruits (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

4. Discussion

The fact that Ψ_{pd} values for T0 plants were high and near constant (Fig. 2) suggested that irrigation water requirements in control quince plants were adequately covered during the experimental period (Fig. 1). In contrast, although the water stress in T1 plants developed slowly (0.019 MPa day^{-1}), the duration of the water withholding period (42 days) led to a substantial water stress level (Ψ_{pd} = −1.27 MPa) (Fig. 2). When T1 plants recovered, the Ψ_{pd} values rapidly increased (0.053 MPa day^{-1}), as has been observed in other fruit trees subjected to a strong water stress (Cruz et al., 2012; Mellisho et al., 2012; Galindo et al., 2016).

The water deficit achieved in T1 plants during the middle of the linear fruit growth phase indicated that this is a critical period for the quince marketable yield. This decrease in marketable yield (kg fruit/ tree) was mainly due to decreased fruit size. Accepting that there is a direct relation between fruit turgor and growth, it is assumed that during the water stress period, T1 fruits lost turgor with a subsequent decrease in the fruit growth rate, which made it difficult for the full fruit size to be recovered when irrigation was restarted (Dell'Amico et al., 2012; Galindo et al., 2016, 2018; Rodríguez et al., 2018).

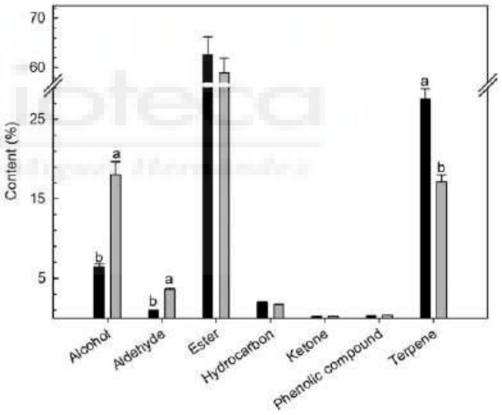

Fig. 4. Chemical families of the volatile compounds (results from Table 6) in T0 (black bars) and T1 (gray bars) quince fruits. Bars for a compound with different letters indicate significant differences at $p \leq 0.05$

Several authors have observed that when the moisture content decreases, for example in jujube or plum fruits, fruit firmness increased as a consequence of a gummy and cork-like jujube flesh structure (Wu et al., 2012) or stiff plum flesh (Salvador et al., 2003). However, quince fruit firmness did not change despite the decrease in fruit moisture content, probably because this decrease in fruit moisture in T1 fruits was not strong enough to cause severe change or damage in quince flesh structure.

The high content of TPC in fruits from both irrigation treatments (Table 5) suggested that quinces, regardless of the fruit water status, can be used as an important source of natural antioxidants.

Table 6

Volatile compounds (mg kg^{-1} dry weight, dw) and descriptors (SAFC, 2014) of quince fruits as affected by irrigation treatments

Compound	Chemical family	Retention index		Descriptors		Concentration (mg kg^{-1} dw)	
		Exp	Lit		ANOVA	T0	T1
Ethyl acetate	Ester	706		Anise; pineapple	*[a]	37.7a[c]	5.1b
Ethyl propanoate	Ester	748			**	35.1a	6.5b
Propyl acetate	Ester	751	695	Celery; pear	NS	14.7	4.6
Ethyl isobutyrate	Ester	779	738	Citrus; strawberry	NS	7.9	5.6
2-Methyl-1-butanol	Alcohol	782	730	Onion	**	68.1b	101a
2-Methyl-2-butenal	Aldehyde	787	722	Green, fruity	NS	19.9	16.7
Isobutyl acetate	Ester	793	743	Apple; banana	NS	7.3	3.2
2-Methyl-2-buten-1-ol	Alcohol	811	766	Herbaceous	NS	13.1	17.6
Ethyl butyrate	Ester	814	774	Banana; pineapple	*	71.7 a	40.4 b
Butyl acetate	Ester	829	786	Banana; green	***	214b	478a
Ethyl 2-methylbutyrate[b]	Ester	862	835	Apple; green; plum	*	138a	53.3b
Ethyl crotanoate = Ethyl 2-butenoate	Ester	866	819		NS	23.6	13.5
Isoamyl acetate	Ester	899	850	Banana; pear; sweet	*	241b	297a
t-2-Hexenal	Aldehyde	901	845	Almond, vegetable	***	33b	137a
1-Hexanol	Alcohol	908	860	Green, herbaceous	***	146b	496
Amyl acetate	Ester	933	880	Banana; earthy	NS	6.3	11.6
Prenyl acetate		940	895	Apple; lavender	NS	57.6	48.2
α-Pinene	Terpene	942	903	Woody	*	17.3b	38a
Ethyl tiglate	Ester	960	919	Caramel; fruity	*	435a	246b
Ethyl hex-4-enoate[b]	Ester	1008	979		NS	40.3	35.7
1-Heptanol	Alcohol	1009	957	Green, nutty, citrus	**	9.9b	19a
Ethyl hexanoate	Ester	1016	979	Apple, pineapple	*	764b	756a
6-Methyl-5-hepten-2-one	Ketone	1024	972	Green; oily	NS	16.8	13.9
(Z)-3-Hexenyl acetate	Ester	1028	987	Banana; vegetable	*	71.7b	80.7a
Hexyl acetate	Ester	1032	991	Fruity; apple; floral	**	138b	218a
Octanal	Aldehyde	1038	982	Honey; fruity; citrus	NS	23.6	21.8
Limonene	Terpene	1041	1009	Lemon, orange	***	43.5 b	288a
Propyl tiglate	Ester	1058	1020		NS	17.3	13.5
2-Ethyl-1-hexanol	Alcohol	1068	1016	Rose	NS	28.8	30.6
Benzenemethanol	Phenolic compound	1108	1053	Berry, cherry, citrus	NS	22	16.7
1-Octanol	Alcohol	1111	1060	Orange; floral	NS	40.3	39
Ethyl heptanoate	Ester	1116	1080	Berry; pineapple	*	141b	178a
IS = NONANAL	Aldehyde	1141	1087		NS		
Methyl octanoate	Ester	1144	1105	Green; citrus; fruity	**	105b	148a
Ethyl 3-hydroxyhexanoate	Ester	1178	1106	grape; smoky; citrus	NS	11	0.5
Benzyl acetate	Ester	1207	1159	Fruity; floral; sweet	NS	41.9	44.1
Ethyl (Z)-4-octenoate+1-Nonanol		1212	1170		*	273b	279a
Ethyl octanoate	Ester	1216	1176	Fruity; apricot; floral	***	3366a	1739b
Decanal	Aldehyde	1244	1184	Floral; citrus; sweet	NS	13.6	13.9
Ethyl-(E)-2-octenoate	Ester	1274	1225		NS	29.3	20.9
Tetradecane	hydrocarbon	1275	1300		NS	36.1	21.8
1,2-Benzisothiazole		1286	1221		NS	48.7	34.3
Propyl octanoate	Ester	1313	1277		*	51.8a	22.3b
α-Copaene	hydrocarbon	1395	1359		NS	38.2	27.4
Ethyl (E)-4-decenoate	Ester	1402	1361		NS	14.1	7.4
Ethyl (Z)-4-decenoate	Ester	1411	1361		NS	22.5	6.5
Ethyl decanoate	Ester	1415	1378	Grape; oily; pear	**	149a	39.9b
β-Caryophyllene	Terpene	1451	1409	Spicy; woody	NS	51.3	40.8
E,E-α-Farnesene	Terpene	1517	1485		***	2853a	1076b
Elemene	Terpene	1531	1494		NS	18.8	18.1
Δ-Cadinene	hydrocarbon	1539	1504		NS	111	86.7
(Z,Z)-α-Farnesene	Terpene	1541	1500	Apple, fruity	NS	33	25.5
Ethyl (E)-9-dodecenoate[b]	Ester	1595			*	119a	41.7b
Ethyl (Z)-4-decenoate[b]	Ester	1608			*	105a	41.7b
Ethyl dodecanoate	Ester	1615	1578	Floral, fruity	*	101a	36.2b
TOTAL						10538a	7503b

a NS = not significant at $p \le 0.05$; *,**, and ***, significant at $p \le 0.05$, 0.01, and 0.001, respectively.

b Tentatively identified (only identified by retention indexes and NIST spectral database, 2000).

c Values followed by the same letter, within the same row, were not significantly different ($p \le 0.05$).

This was even more interesting considering that quince fruits were harvested at commercial ripening (and can thus be considered as fully ripe), and that, according to Remorini et al. (2008), a decrease in TPC is a characteristic of the progress of ripening in fruits, and can be attributed to changes such as hydrolysis of glycosides, the oxidation of phenols by polyphenol oxidases and polymerization of free phenols.

The antioxidant capacity of quince flesh extracts was assessed using ABTS+, DPPH•, and FRAP assays to evaluate the different modes of action of antioxidants (Prior and Cao, 1999; Huang et al., 2005). In this sense, the significant correlation between AA-ABTS+ and TPC values indicated that TPC content determine the AA-ABTS+ levels (Table 5), whereas FRAP changes by water deficit effect were not directly mediated by TPC changes in this particular vegetal material. In addition, the constancy of AA-DPPH• in response to water stress could be due to the fact that this test only considers lipophilic AA (Kuskoski et al., 2005), and is less sensitive than AA-ABTS+ and AA-FRAP for measuring antioxidant defences (Redha et al., 2014).

In accordance with the quince fruit sensory analysis, the perceived increase in peel colour intensity (fruits were less green) in in the middle of linear fruit growth phase could be used as a tool to improve T1 quinces (Fig. 3) agreed with the instrumental colour data, and, more precisely, with data on the green-red coordinate, a* (Table 3). Regarding the texture attributes, it is important to underline that corkiness and fibreness in T1 quince fruits decreased by around 50%, whereas crunchiness increased (Fig. 3). Thus, water stress improved quince texture, because fruit corkiness and fibreness are two of the main handicaps for its consumption as fresh fruit (Szychowski et al., 2014). According to the volatile profile of quince fruits (Table 6), the high number of compounds identified confirmed the high odour and aroma intensity of these fruits. Water deficit induced important changes in an important number of these identified volatile compounds (Table 6).

These changes were characterized by decreases in the contents of ethyl octanoate and E,E-α-farnesene, which have pear, pineapple and apple sensory descriptors and increases in butyl acetate (banana), 1-hexanol (herbaceous) and ethyl hexanoate (apple and pineapple). An important fact is that even though the two most abundant compounds were found in T0, there were five compounds above 400 mg kg^{-1} dw in T1 quinces as compared to only 3 in T0 samples.

5. Conclusions

The linear fruit growth phase was seen to be a critical phenological period in quince because withholding irrigation water in the middle of this period decreased the fruit weight, size and moisture content, and, as a consequence, marketable yield. In addition, this period was also clearly critical for the chemical characteristics of quince fruit because water deficit leads to important changes in most of the volatile compounds identified, increasing the total soluble solids content and total polyphenolic content and decreasing antioxidant activity. The high levels of total polyphenolics, regardless of the fruit water status, suggested that quinces can be used as an important source of natural antioxidants. Moreover, the fruit sensory analysis indicated that water deficit during fruit growth increases peel colour intensity and improves fruit flesh texture because the main drawbacks for its consumption as fresh fruit (corkiness and fibreness) severely decreased, whereas crunchiness increased.

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7.5 Publication 5 (Literal Transcription)

Water stress at the end of the pomegranate fruit ripening stage produces earlier harvest and improves fruit

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Water stress at the end of the pomegranate fruit ripening stage produces earlier harvest and improves fruit quality

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ABSTRACT

Pomegranate (Punica granatum L.) is a drought tolerant crop, which thrives in the face of scarce water resources, this fact underlines the importance of determining the optimum harvest time to improve the quality of pomegranate fruits. This research was focused on the crop responses to drought stress during the phenological period of fruit ripening. Special attention was paid to the effects on plant productivity of water restrictions during fruit ripening and whether such restrictions have secondary effects on fruit characteristics and composition. Control plants were irrigated above crop water requirements while deficit irrigation treatments were irrigated as control plants except for 6 days (fruit late ripening), 15 days (second half fruit ripening), 25 days (fruit ripening), and 36 days (end fruit growth and late ripening) before harvest, when irrigation was withheld. The results indicated that the water stress integral, calculated from leaf conductance, leaf water potential, stem water potential and fruit water potential data, differed in their assessment of the cumulative water deficit reached by the plants. Also, pomegranate fruit ripening was confirmed as a critical period because irrigation is clearly essential during most of this phenological period to achieve maximum yield. Moreover, a very short period of irrigation restriction (around 6 days) at the end of ripening period comes early harvest time, saves irrigation water, enhances the bioactive compounds content (anthocyanins, phenolic compounds, punicalagin and ellagic acid) and increases the price of the fruit without affecting marketable yield and fruit size. This suggests that the sensitivity to water stress during such a critical phenological period is not constant and/or that for productivity to be adversely affected it is necessary to exceed a threshold level of water stress.

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1. Introduction

Pomegranate (Punica granatum L.) plants are equipped with xeromorphic characteristics such as a high leaf relative apoplastic water content and the ability to develop complementary stress avoidance and stress tolerance mechanisms to confront drought (Rodríguez et al., 2012). That means that it is able to thrive in arid and semi-arid areas, even under desert conditions (Aseri et al., 2008). Nevertheless, to reach optimal growth, yield and fruit quality for commercial production, the crop requires regular irrigation throughout the dry season (Prasad et al., 2003; Shaliendra and Narendra, 2005; Sulochanamma et al., 2005; Holland et al., 2009).

The commercial production of pomegranate in the Mediterranean Basin is characterized by high quality fruits (Stover and Mercure, 2007; Holland et al., 2009) with high bioactive compounds content (Gil et al., 2000; Poyrazoğlu et al., 2002; Mena et al., 2011) and a correspondingly high antioxidant capacity and beneficial health effects (Lansky and Newman, 2007).

All Mediterranean agrosystems must cope with water scarcity, and any policy involving greater use of the water (Pereira et al., 2002). In this sense, pomegranate farming must be directed towards the use of deficit irrigation strategies, allowing significant water savings, and the profitable production of high quality fruits. Sustained deficit irrigation (SDI) is an irrigation strategy in which the amount of water applied at any moment of the season is lower than that needed to satisfy the full crop water requirements (English and Raja, 1996). Regulated deficit irrigation (RDI) is another irrigation strategy designed to save water while having a minimum impact on yield and fruit quality (Goldhamer, 1989; Naor, 2006). This requires precise knowledge of the crop response to drought stress during different phenological periods when adverse effects on productivity are minimal (non-critical periods) or maximal (critical periods).

Reports on the effect of irrigation management on pomegranate fruit yield and quality are very scarce. The first results indicated that it is possible to control the desired ripening time in pomegranates by applying different irrigation regimes (Sonawane and Desai, 1989). Also, Prasad et al. (2003), Shaliendra and Narendra (2005), and Sulochanamma et al. (2005) showed that irrigation has a positive effect on pomegranate vegetative growth, yield, and fruit weight. Recently, Galindo et al. (2014a) indicated that SDI applied throughout the pomegranate season reduces total yield per tree, the number of fruits per tree and the size of the fruits; however, such a strategy can advance the availability of fruits from late flowerings, which despite their smaller size are of high interest for the pomegranate industry due to their very high content of bioactive compounds. In contrast, Mellisho et al. (2012) concluded that SDI, under moderate water stress, showed some changes in colour and chemical characteristics, which reflected earlier ripening. However, Mena et al. (2013) indicated that pomegranate juice from trees submitted to SDI that produces severe water stress levels was of lower quality and less healthy than that from fully irrigated trees. On the other hand, Peña-Estévez et al. (2015) concluded that pomegranates from SDI trees had good sensory qualities, a higher content of most bioactive compounds, and suffered less chilling injury during cold storage and shelf-life than fully irrigated fruits. Recently, Laribi et al. (2013) showed that pomegranates from SDI trees, submitted to mild water stress during flowering and fruit set and more severe water stress during the linear phase of fruit growth and ripening, had a redder peel and higher level of total soluble solids in the juice.

To the best of our knowledge, there has been no scientific study evaluating the response of pomegranate to RDI, applying full irrigation in all the critical periods and deficit irrigation during the non-critical periods. However, Intrigliolo et al. (2013) and Laribi et al. (2013) studied pomegranate response to severe irrigation water restrictions applied during the phenological periods of (i) flowering, fruit set and early fruit growth, (ii) linear fruit growth, and (iii) the last part of fruit growth and ripening. These authors concluded that the phenological period comprising flowering and fruit set could be regarded as noncritical from the yield point of view. Moreover, Laribi et al. (2013) concluded that irrigation water restriction during pomegranate fruit growth and ripening enhances peel red colour intensity and total soluble solids in the juice, while irrigation water restriction during linear fruit growth period increased the concentration of many bioactive compounds in the juice, such as anthocyanins, that could be related to health and taste.

For this reason, the aim of this research was to (i) clarify whether the pomegranate fruit ripening phenological stage is a critical or noncritical period from the yield point of view, (ii) whether pomegranate yield response to water restriction during ripening depends on the exact point at which water stress takes place, and (iii) evaluate whether water restrictions during the ripening stage have secondary effects on fruit characteristics and composition.

2. Material and methods

2.1. *Plant material, experimental conditions and treatments*

The experiment was carried out in 2013 in a pomegranate (Punica granatum L.) orchard located near the city of Alhama de Murcia (Spain) (37°47′N, 1° 25′W). The trees were own-rooted 15 years old Mollar de Elche cultivar and the tree spacing was 3 m × 5 m. The soil of the orchard is a moderately saline silt loam (Hyposalic Calciorthid), with moderate lime content, very low organic matter content, low cationic exchange capacity, high available phosphorus levels and low available potassium. The irrigation water used had an electrical conductivity of between 0.9 and 1.3 dS m^{-1}. The chloride (Cl−) concentration in the irrigation water ranged from 67 to 78 mg L^{-1} during the experimental period. Pest control and fertilization practices were those usually used by local growers, and no weeds were allowed to develop within the orchard.

Micrometeorological data, namely air relative humidity, air temperature, solar radiation, rainfall and wind speed 2 m above the soil surface, were collected by an automatic weather station located near the experimental site. Mean daily air vapour pressure deficit (VPDm) and daily ETo, using the Penman–Monteith equation, were calculated as described by Allen et al. (1998).

During the growing season, control plants (T0) were irrigated above crop water requirements (123% ETc) in order to ensure non-limiting soil water conditions. Irrigation was performed daily during the night using a drip-irrigation system with a lateral pipe parallel to each tree row and 3 emitters per tree, each delivering 4 L h^{-1}. In-line water meters were used to measure the water supplied to each experimental unit. T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277–283, fruit late ripening), 15 (DOY 268–283, second half fruit ripening), 25 (DOY 258–283, fruit ripening) and 36 (DOY 247–283, end fruit growth and late ripening) days before harvest (DOY 283), respectively, when irrigation was withheld. The total amount of water received by each treatment during the experimental period (DOY 247–283) was 128, 110, 86, 49 and 0 mm for T0, T1, T2, T3 and T4 treatments, respectively, without considering precipitation (basically the 84 mm that fell on DOY 271).

2.2. *Plant water status*

The water relations of the leaves and fruits were measured at midday (12 h solar time). Fruits and fully expanded leaves from the south-facing side and middle third of the tree of four trees per treatment were selected for measurements. Midday leaf conductance (g_{leaf}) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday fruit water potential Ψ_{fruit}), midday leaf water potential (Ψ_{leaf}), and midday stem water potential (Ψ_{stem}) were measured in two fruits or two leaves similar to those used for g_{leaf} using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA) (McFadyen et al., 1996; Galindo et al., 2014b). Leaves for Ψ_{stem} measurements were enclosed in a small black plastic bag covered with aluminium foil for at least 2 h before the measurements were made.

In order to assess the cumulative effect of the water deficit, the water stress integral (SI) was calculated from the g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data according to the expression proposed by Myers (1988).

$$SI_A = \left| \sum (\bar{A}-H)n \right|$$

where A can be g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} and $\bar{A}$ is the average g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} value for any interval, H is the maximum value measured during each interval and n is the number of days in the interval.

2.3. *Fruit physico-chemical analysis*

In order to study any changes in pomegranate fruit due to the irrigation treatments, the samples from each treatment were picked on 16 October (DOY 283). Forty-eight fruits per treatments were harvested (four trees for treatments and twelve fruits per tree). All the fruits were transported to the laboratory and analyses were performed immediately. For each fruit, the following parameters were measured: maximum width or equatorial diameter, ED (mm), and fruit length from calyx to base, FL (mm), using a digital calliper/calliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK) with 0.01 mm accuracy; fruit weight, FW (g) using a precision weighing balance (Mettler AJ50, Goettingen, Germany) with an accuracy of 0.0001 g. Then, the fruits were peeled by hand and the arils were weighted, homogenised, and half of the arils was squeezed, between two layers of muslin cloth, to extract the complete juice. The juice was centrifuged (1200g) at 4 °C and stored at −70 °C until the chemical analyses were conducted total soluble solids (TSS), titratable acidity (TA) and (pH), and the other half of the arils were immediately frozen in liquid nitrogen and later freeze dried in an Alpha 2–4 freeze drier (Alpha 2–4; Christ, Osterode am Harz, Germany) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. At the end of freeze-drying, the samples were powdered and packed under vacuum. Antioxidant activity (AA), total polyphenol content (TPC), total anthocyanin content (TAC), α-punicalagin, β-punicalagin and ellagic acid were analysed.

2.3.1. *Total soluble solids, pH and total titratable acidity.*

TSS were measured with a digital Atago refractometer (model N-20; Atago, Bellevue, WA) at 20 °C with values being expressed as °Brix. The TA and pH were determined by acid–base potentiometer (877 Titrino plus; Metrohm ion analyses CH9101, Herisau, Switzerland), using 0.1 N NaOH up to pH 8.1; values were expressed as g citric acid L^{-1}. Finally, the maturity index (MI), which is a ratio of TSS to TA, was also calculated for each sample.

2.3.2. *Colour determination.*

Pomegranate juice colour was determined and measured in glass cells of 2 mm path length (CT-A22). A Minolta CR 2000 colorimeter (Osaka, Japan) was used and results were expressed in the CIE *L*, a*, b** system, and the mean values of lightness (CIE *L**), red/greenness (CIE *a**) and blue/yellowness (CIE *b**) coordinates for each juice were calculated. The objective colour was calculated as chromaticity or chroma [$C^* = (a^{*2} + b^{*2})^{½}$] and hue angle [$H° = \tan^{-1}(b^*/a^*)$].

2.3.3. Total polyphenols content, total anthocyanin content and antioxidant activity.

Total phenolic compounds (TPC) were determined using Folin–Ciocalteu reagent. Briefly, an aliquot of filtered juice was diluted with 0.4 mL of phosphate buffer (50 mmol L^{-1}, pH = 7.8). Folin–Ciocalteu reagent (2.5 mL) was added and the content of the flask was mixed thoroughly. After 8 min, an Na2CO3 solution (10 mL, 10%, w/v) was added and the samples were incubated in a water bath at 50 °C for 5 min. The resulting blue colour was measured spectrophotometrically at 760 nm. The concentration of the total polyphenol compounds in juice was determined by comparison with the absorbance of gallic acid at different concentrations. Results were expressed as mg of gallic acid (GAE) L^{-1} of juice.

The total anthocyanin content (TAC) was determined by a pH differential method with two buffer systems, sodium acetate buffer, pH 4.5 (0.4 mol L^{-1}) and potassium chloride buffer, pH 1.0 (0.025 mol L^{-1}) (Giusti et al., 1999). Pomegranate juice (0.4 mL) was mixed with 3.6 mL of the corresponding buffers and read against water as blank at 510 nm and 700 nm. The absorbance (A) was calculated as $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5.}$ The total anthocyanin content was calculated by following the equation TAC =(A × MW × DF × 100/MA), where A is the absorbance, MW is the molecular weight (449.2), DF is the dilution factor (10), and MA is the molar absorptivity of cyaniding-3-glucoside (26.900). The result was expressed as mg cyaniding-3-glucoside (C3G) L^{-1} of pomegranate juice.

For the total antioxidant activity (TAA), a methanol extract was prepared with each sample to be analysed. Freeze-dried fruits (0.5 g) were mixed with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, sonicated at 20 °C for 15 min and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 15,000 rpm for 10 min. The radical scavenging activity was evaluated using the DPPH radical (2,2-diphenyl-1-picrylhydrazyl). Briefly, 10 mL of the supernatant were mixed with 40 mL of MeOH and added to 950 mL of DPPH solution. The mixture was shaken vigorously and placed in a dark room for 10 min. The decrease in absorbance was measured at 515 nm using a UV–VIS Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves, in the range 0.01–5.00 mmol Trolox L^{-1}, were used for the quantification of antioxidant activity, showing good linearity ($r^2 \geq 0.998$). Results are expressed as mM Trolox.

2.3.4. Punicalagin isomers and ellagic acid.

The punicalagin (isomers α and β) and ellagic acid contents were determined in freeze-dried fruits (0.3 g) diluted with 7 mL of MeOH/ water (80:20, v/v) and 1% acetic acid and then centrifuged at 15,000 rpm for 20 min. Supernatants were filtered through a 0.45 μm Millipore filter and then injected into a Hewlett-Packard series 1200 HPLC equipped with a diodearray detector. Each sample (20 mL) was analysed on a LiChroCART 100 RP-18 reversed-phased column (250 mm x 4 mm, particle size, 5 μm; Merck, Darmstadt, Germany) equipped with a C18 precolumn (LiChrospher 100 RP-18, 5 mm; Merck, Darmstadt, Germany) using a mobile phase of 1% acetic acid in ultra-high purity deionised water (solvent A) and 1% acetic acid in MeOH (solvent B). Elution was performed at a flow rate of 1 mL min^{-1} using a gradient starting with 1% B for 5 min, and increasing to 60% B for 40 min. Punicalagin (α and β) and ellagic acid were detected at 360 nm.

To confirm their identification, absorption spectra and retention times were compared with those obtained from chemical standards. Standard curves for pure punicalagins (Chengdu Biopurify Phytochemicals Ltd., Sichuan, China), with a concentration range of 0.05–0.80 g L^{-1}, as well as for ellagic acid (Tocris Bioscience, Ellisville, MO), with a concentration range of 0.0025–0.0200 g L^{-1}, were used for quantification. The results for individual isomer punicalagin (α and β) and ellagic acid are expressed as mg L^{-1}.

2.4. Statistical design and analysis

The design of the experiments was completely randomized with four replications, each replication consisting of three adjacent rows, each with thirteen trees. Measurements were taken on the inner most trees of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), while the other trees served as border trees. Data were analysed using Statgraphics 5.1 for Windows (Statpoint Technologies, Warrenton, VA, USA). A basic descriptive statistical analysis was followed by an analysis of variance (ANOVA) test for means comparisons. Fisher's Least Significant Difference (LSD) procedure at a 95.0% confidence level was used to discriminate among the means (Multiple Range Test). Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

3.1. Climate and plant water status

The experimental conditions were semiarid, characterized by a VPDm ranging from 0.33 to 1.87 kPa, average daily maximum and minimum air temperatures of 28.0 and 14.8 °C, respectively, and accumulated ETo of 162 mm. Total rainfall was 88.4 mm: DOY 270 (3.5 mm), DOY 271 (84 mm) and DOY 272 (0.9 mm)

Table 1 describes the cumulative effect of the irrigation treatments on the pomegranate trees using SI_{gleaf}, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$ values, whose values in the different irrigation treatments tended to increase with the number of days irrigation was withheld. However, SI_{gleaf}, $SI_{\Psi stem}$ and $SI_{\Psi leaf}$ values in T0, T1 and T2 were statistically equivalent, whereas $SI_{\Psi fruit}$ values in these treatments showed differences, the $SI_{\Psi fruit}$ values in T2 being significantly higher than in T0 but similar to that in T1, which was also similar to that in T0 (Table 1). Moreover, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$ values in T3 and T4 plants presented similar values, whereas SI_{gleaf} values in T4 plants were significantly higher tan those in T3 plants (Table 1).

Table 1
Effect of irrigation treatments on leaf conductance (SI_{gleaf}, mmol m−2 s−1 x day), stem ($SI_{\Psi stem}$, MPa x day), leaf ($SI_{\Psi leaf}$, MPa x day) and fruit ($SI_{\Psi fruit}$, MPa x day) water stress integral

Treatment	SI_{gleaf}	$SI_{\Psi stem}$	$SI_{\Psi leaf}$	$SI_{\Psi fruit}$
T0	5434.5c	10.5b	27.6c	8.5c
T1	5911.5c	15.0b	29.1c	15.1bc
T2	6414.0c	21.5b	31.1bc	22.7b
T3	9784.0b	49.5a	49.2ab	43.7a
T4	15215.3a	62.8a	53.5a	52.9a

Means within a column that do not have a common letter are significantly different by LSD0.05 test.

3.2. *Yield and fruit physical characteristics*

The marketable yield of pomegranates was significantly reduced by the withholding of water: the longer the period without water, the lower the marketable yield (Table 2). In this sense, control (T0) plants and those from which water was withheld during late ripening (T1) showed similar yields (56.8 and 55.5 kg tree^{-1}, respectively) but higher than those of T2, T3 and T4 plants (35.2, 28.9 and 17.8 kg tree^{-1}, respectively), which were similar among themselves. Harvested pomegranate fruits affected by peel cracking and/or peel splitting (data not shown) were significantly higher in T3 and T4 (30.5 and 31.9 kg tree^{-1}, respectively), which had longer water withholding periods and lower in T0 plants (7.6 kg tree^{-1}) whereas T2 plants showed intermediate values (19.8 kg tree^{-1}) and similar to T1 plants (14.7 kg tree^{-1}), which at the same time were statistically similar to T0 plants.

Table 2
Effect of irrigation treatments on marketable pomegranate fruit yield (MY, kg tree^{-1}), average fruit weight (FW, g), fruit equatorial diameter (ED, mm), and fruit length (FL, mm).

Treatment	MY	FW	ED	FL
T0	56.8a	293a	86.9a	75.0a
T1	55.5a	258ab	80.4b	69.7b
T2	35.2b	252b	81.4b	71.4ab
T3	28.9b	249b	81.6b	69.8b
T4	17.8b	253b	78.1b	67.2b

Means within a column that do not have a common letter are significantly different by $LSD_{0.05}$ test

The effect of withholding water during the different phases of fruit ripening on average fruit weight was similar to the effect on fruit yield, with the characteristic that T1 fruit weight (258 g) was statistically equivalent to that measured in T0, T2, T3 and T4 (293, 252, 249 and 253 g, respectively) (Table 2). Fruits from T0 plants had the highest equatorial diameter (86.9 mm), whereas no differences in this value were observed in the other water withheld treatments (from 78.1 up to 81.6) (Table 2). Additionally, fruits from T1, T3 and T4 plants showed significant lower fruit length (69.7, 69.8 and 67.2 mm, respectively) than fruits from T0 plants (75.0 mm); whereas fruits from T2 plants showed an intermediate response with a fruit length similar to that observed in the other four treatments (71.4 mm) (Table 2).

A significant effect of water stress on pomegranate peel and juice colour was observed (Table 3). Thus, *L**, *b** and *H*° values of the peel tended to decrease with accumulated water stress effect (from 64.2 to 55.1, from 31.2 to 26.8 and from 50.1 to 38.2, respectively), while a* and *C** values increased from 26.5 to 34.6 and from 41.6 to 44.3, respectively, leading to a fruit peel with higher redness and darkness values (Table 3). Coordinates *L**, *a**, *b**, *C**, and *H*° values of the pomegranate juice showed a tendency to increase as the days of water reduction increased, but no significant differences in *L**, *a**, *b** and *C** values were found between T0, T1 and T2 and in *H*° values between T0, T1, T2 and T3 (Table 3).

Table 3
Effect of irrigation treatments on pomegranate peel and juice lightness (CIE *L**), red/ greenness (CIE *a**), blue/yellowness (CIE *b**), chroma (*C**) and hue angle (*H*°) values.

Treatment		*L**	*a**	*b**	*C**	*H*°
Peel	T0	64.2a	26.5b	31.2a	41.6b	50.1a
	T1	60.8ab	30.7a	30.5a	43.9a	45.3ab
	T2	60.1b	32.0a	30.1ab	44.5a	43.7abc
	T3	57.2bc	33.5a	27.6bc	43.9a	40.1bc
	T4	55.1c	34.6a	26.8c	44.3a	38.2c
Juice	T0	32.4b	8.3b	2.3b	8.6b	14.8b
	T1	33.1ab	10.5ab	3.1ab	10.9ab	16.5ab
	T2	33.1ab	9.6ab	2.7ab	9.9ab	15.3ab
	T3	33.9a	11.7a	3.6a	12.3a	17.2ab
	T4	33.7a	11.8a	3.9a	12.5a	18.2a

Means within a column for each fruit part that do not have a common letter are significantly different by LSD0.05 test

3.3. *Fruit chemical characteristics*

TSS values in T1, T2, and T3 fruits were similar and lower (~17° Brix) than in T0 fruits (17.8° Brix), while the TSS value in T4 fruits showed an intermediate value (17.5° Brix) similar to that measured in T0 and T1 and T3 fruits (Table 4). TA values in T0, T1, T2, and T4 fruits were similar (ranging between 2.11 and 2.17 g citric acid L^{-1}); while the TSS in T3 (2.23 g citric acid L^{-1}) fruits was higher than in T1 and similar to that observed in T0, T2 and T4 fruits. Moreover, non-significant differences among treatments were observed in pH values, with MI values showing a similar trend to that mentioned for TA values, with non-significant differences among T0, T1, T2 and T4, but with a T3 value lower than in T0, and similar to those of the other treatments (Table 4).

Table 4
Effect of irrigation treatments on pomegranate juice total soluble solids (TSS, °Brix), ti- trable acidity (TA, g citric acid L^{-1}), pH and maturity index (MI, TSS/TA).

Treatment	TSS	TA	pH	MI
T0	17.8a	2.17ab	4.9a	82.2a
T1	17.1bc	2.11b	4.8a	81.3ab
T2	17.1c	2.15ab	4.9a	79.6ab
T3	17.2bc	2.23a	4.7a	77.1b
T4	17.5ab	2.16ab	4.7a	81.2ab

Means within a column that do not have a common letter are significantly different by LSD$_{0.05}$ test.

TPC progressively decreased as the number of water withholding days increased (Table 5), reaching minimum values in T2, T3, and T4 fruits (1945, 1534, and 1589 mg GAE L^{-1}, respectively, with no significant differences among these three treatments). In contrast, withholding water did not affect the TAA values, while its effects on TAC, α-punicalagin β-punicalagin and ellagic acid were not very pronounced, inducing (i) a significant TAC increase only in T1 fruits (123.1 mM Trolox), (ii) a slight but significant ellagic acid increase in fruits under water stress, although no significant differences among T1, T2, T3, and T4 (19.6, 19.5, 19.5 and 19.3 mg L^{-1}) were observed, and the value in T4 fruits (19.3 mg L^{-1}) was also similar to that in T0 fruits (19.0 mg L^{-1}), (iii) no significant differences in α-punicalagin values among treatments, except in T1 fruits (184.2 mg L^{-1}), which showed higher values than T3 and T4 (162.2 and 157.7 mg L^{-1}), but similar values to those in T0 and T2 (168.7 and 169.5 mg L^{-1}), and (iv) a significant increase in β-punicalagin in T1 and T2 fruits, which reached 172.0 and 174.2 mg L^{-1}, respectively (Table 5).

4. Discussion

A detailed analysis of the effects of the irrigation water withholding treatments on plant and fruit water status were described in a previous manuscript from our research network (Galindo et al., 2014b). Bearing in mind the results from that article and those shown in Table 1, it is clear that, in spite of the rainfall events (occurring on DOY 271), the cumulative water stress tended to increase with the number of days irrigation was withheld, the treatments in which irrigation was withheld during late fruit ripening (T1) and during the second half of fruit ripening (T2) producing a similar and moderate water stress level and a more pronounced water stress level being observed in the treatments in which irrigation was withheld during ripening (T3) and at the end of fruit growth and ripening (T4). In addition, it is important to highlight the fact that SI_{gleaf}, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$ values showed some differences in describing the cumulative water deficit reached by the plants. $SI_{\Psi fruit}$ was the most reliable indicator to detect differences between the treatments at moderate water stress (T0 and T2), while SI_{gleaf} was the only indicator able to detect differences between the treatments at more pronounced water stress levels (T3 and T4).

The decrease in fruit yield in water stressed plants during the second half of fruit ripening (T2) and during fruit ripening (T3) (Table 2) confirmed the hypothesis that fruit ripening is a critical period from the yield point of view (Intrigliolo et al., 2013; Laribi et al., 2013). However, the fact that plants that were water stressed only at fruit late ripening stage (T1) showed similar marketable yield and fruit size to fully irrigated plants (T0) mean to clarify some aspects of the concept of phenological critical period (Goldhamer, 1989; Naor, 2006). In this sense, it is probable that sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Whatever the case, although pomegranate trees are able to withstand severe drought conditions (Rodríguez et al., 2012; Galindo et al., 2014b), irrigation was essential during most of the ripening stage to achieve optimum yield. According to Galindo et al. (2014b), the decrease in the marketable yield in T2, T3 and T4 plants was due mainly to the incidence of the fruit cracking and/or fruit splitting disorders and to the decrease in fruit size, which can be attributed to a loss of fruit turgor, because a direct relation between turgor and growth has been found in many studies (Serpe and Matthews, 2000; Matthews and Shackel, 2005).

In agreement with the results reported by Laribi et al. (2013) in pomegranate and Collado-González et al. (2014) in jujube fruits, withholding irrigation water during the ripening phase increases redness and darkness of the fruit peel (Table 3). In this sense, the absence of data for peel pigments prevents any conclusion concerning whether the changes in peel colour were due to anthocyanin accumulation. Nevertheless, a negative correlation between lightness and pigment content is known, because as pigment levels increase, more light is absorbed, and lower values of luminosity are recorded. Moreover, considering that fruit peel from T1 plants, in which irrigation was withheld during late ripening for only 6 days, was also redder and darker than in fruits from T0 (Table 3), it is possible to rule out higher fruit exposure to sunlight as the only cause of colour changes (Gelly et al., 2004)

Table 5
Effect of irrigation treatments on pomegranate juice total polyphenols content (TPC, mg GAE L^{-1}), total anthocyanin content (TAC, mg L^{-1}), total antioxidant activity (TAA, mM Trolox), α- punicalagin, β-punicalagin, and ellagic acid (mg L^{-1}).

Treatment	TPC	TAC	TAA	α-punicalagin	β-punicalagin	Ellagic acid
T0	3133a	69.5b	12.1a	168.7ab	164.5b	19.0b
T1	2681b	123.1a	13.3a	184.2a	174.2a	19.6a
T2	1945c	76.1b	13.3a	169.5ab	172.0a	19.5a
T3	1534c	75.1b	11.9a	162.2b	170.2ab	19.5a
T4	1589c	75.1b	12.0a	157.7b	168.7ab	19.3ab

Means within a column that do not have a common letter are significantly different by LSD0.05 test

because a significant reduction in the canopy characteristics is not very likely in only 6 days.

It is important to take remember that the first pomegranate fruits reaching the market fetch higher prices and, in this sense, 'Mollar de Elche' cultivar is often harvested when the peel has a sufficient red colouratiing (Manera et al., 2013). The significant increase in juice colour from T3 and T4 fruits (Table 3) is also very interesting for producers because pomegranate fruit attractiveness is primarily related to colour and taste parameters of the arils and their juice (Borochov-Neori et al., 2009). However, despite the fact that pomegranate colouration in pomegranates is predominantly due to anthocyanins (Shulman et al., 1984), TAC levels in T3 and T4 fruits were similar to that observed in T0 fruits (Table 5). Laribi et al. (2013) showed also similar behaviour in juice from trees submitted to severe water restrictions during the last part of fruit growth and ripening period.

The fact that (i) TAC juice levels increased only in T1 fruits, (ii) TAA levels were similar in juices from the different irrigation treatments, and (iii) redness significantly increased only in T3 and T4 fruit juices (Tables 3 and 5), confirmed the view that juice antioxidant capacity is not linearly correlated with the red colour intensity, meaning that the anthocyanins are not major contributors to the antioxidant capacity exhibited by the pomegranates and their juice (Borochov-Neori et al., 2009). Moreover, the fact that withholding water irrigation decreased TPC levels and did not affect TAA levels (Table 5) does not agree with the linear relationship between soluble phenolic levels and antioxidant capacity indicated by Borochov-Neori et al. (2009), who supported the idea that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this sense, further analysis of fatty acids (Alcaraz-Mármol et al., 2015) and organic acids (Calín-Sánchez et al., 2013) must be conducted to fully understand the antioxidant capacity and bioactivity of pomegranate fruits subjected to deficit irrigation strategies.

The behaviour observed in TSS, TA, pH and MI juice values (Table 4) in response to irrigation withholding was not very clear and showed certain differences with respect to the results indicated by other authors in similar experiments. To be precise, Mellisho et al. (2012) indicated that arils from fruits exposed to water deficit during the second half of the linear fruit growth phase showed very similar overall chemical characteristics to arils from fully irrigated fruits, and Labiri et al. (2013) found a significant increase in TSS and TA levels in pomegranate juice from plants exposed to water deficit during the finaln phase of fruit growth and ripening. Additionally, when the effect of SDI applied throughout the season on pomegranate fruit quality is considered, the results at first sight are ambiguous. Mena et al. (2013) indicated that an SDI strategy that induced severe water stress led to pomegranate juice of lower visual attractiveness and less healthy (more yellowish, lower antioxidant activity and lower total phenolic compound, punicalagin and total anthocyanin contents) than the juice from fully irrigated trees. In contrast, Galindo et al. (2014a) concluded that SDI inducing severe water stress led to fruits with similar bioactive quality but a darker and more intense garnet colour than fully irrigated fruits, bringing the optimal harvest time by about 7–8 days. Also, Mellisho et al. (2012) showed that SDI inducing moderate water stress throughout the season led to changes in colour and chemical characteristics, which reflected earlier ripening.

In this sense, it is well known that water stress influences the content of secondary metabolites in plant tissues, having also contradictory results in other crops. For example, Chaves et al. (2007) reported the substantial accumulation of anthocyanins in grape berries under water stress. In contrast, Kennedy et al. (2000, 2002) showed that osmotic stress had little or no effect on anthocyanin accumulation in grape berries. This, at first sight, confusing relation between water stress and the production of bioactive compounds could be attributed to the fact that most manuscripts are not meticulous when it comes to recording aspects of plant water stress (precise phenological period at which it takes place, water stress rate of development, duration of maximum water stress, incidence of partial recoveries and other aspects) although such information is essential for the characterisation of experimental water stress conditions. In addition, it is essential to underline that is not possible to entablish a linear correlation between water stress and secondary metabolite contents (Mattsson and Haack, 1987; Gobbo-Neto and Lopes, 2007). For this reason, Horner (1990) proposed a quadratic model to predict the concentration of phenolic compounds as a function of plant water status. So, under a mild water stress, CO2 assimilation could be maintained and carbon-based secondary metabolites will probably increase when carbohydrates exceed the amount required for growth. Thus, mild osmotic stress may lead to a reduction in plant growth, accompanied by an increasing concentration of non-nitrogenous secondary metabolites. When water stress increases, stomatal regulation takes place and CO2 assimilation is reduced. In this situation, carbon will be preferentially allocated to the synthesis of primary metabolites to the detriment of the synthesis of secondary metabolites (Mellisho et al., 2012).

5. Conclusion

The present results indicated that the SI calculated from g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data vary as regards their ability to describe the cumulative water deficit reached by plants. $SI_{\Psi fruit}$ was the most feasible indicator for detecting differences between the treatments at moderate water stress levels while SI_{gleaf} was the only indicator able to detect differences between the treatments at higher water stress levels. Moreover, pomegranate fruit ripening is a critical period from the yield point of view because irrigation is essential during most of this phenological period if maximum yields are to be achieved. Nevertheless, the fact that a very short irrigation restriction period (around 6 days) at the end of ripening bring the harvest time forward and so increase pomegranate fruit price, saves irrigation water and enhances the bioactive compound content (anthocyanin, phenolic compounds, punicalagin and ellagic acid) without affecting marketable yield and fruit size suggests that the sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Moreover, the increase in fruits colouration as a result of water stress during fruit ripening may be considered as an interesting aspect because the appeal of pomegranate fruit is directly associated with colour. In spite of this, it is important to note that a very short irrigation restriction (around 6 days) at the end of the ripening period advances the harvest time, increases pomegranate fruit price, saves irrigation water and enhances the bioactive compound contents (anthocyanin, phenolic compounds, punicalagin and ellagic acid). Finally, the results confirmed the hypothesis that there is no a linear correlation between pomegranate water stress and secondary metabolite contents, because mild water stress may lead to a reduction in plant growth and a higher concentration of secondary carbon metabolites, whereas under a more pronounced water stress carbon are preferentially allocated to the synthesis of primary metabolites to the detriment of secondary metabolites.

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7.6 Publication 6 (Open Access)

Effect of preharvest fruit bagging on fruit quality characteristics and incidence of fruit physiopathies in fully irrigated and water stressed pomegranate trees

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Effect of preharvest fruit bagging on fruit quality characteristics and incidence of fruit physiopathies in fully irrigated and water stressed pomegranatetrees

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Abstract

BACKGROUND: This report studied the response of pomegranate fruit under full irrigation (FI) and water stress conditions to bagging with externally glossy, single-layer, cellulosic paper bags, open at the bottom, from the end of fruit thinning to harvest time.

RESULTS: Bagging decreased fruit size and the maturity index, and increased antioxidant activity in FI conditions. Moreover, fruit bagging substantially reduced the incidence of peel sunburn in both irrigation conditions.

CONCLUSION: The delay in fruit growth and ripening as a result of pomegranate fruit bagging is outweighed by the very important commercial benefit in terms of the reduced incidence of peel sunburn and the increase in fruit antioxidant activity.
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Keywords: fruit splitting; fruit sunburn; *Punica granatum*; water deficit

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and, along with the species *Punica protopunica*, it constitutes the Punicaceae family.[1] Pomegranate fruit has been extensively used in medicine by many traditional cultures because it is one of the healthiest fruits in terms of antioxidant activity[2-4] with high anticarcinogenic compound content,[5-7] and antiatherosclerotic effects that are able to reduce blood pressure.[4] Pomegranate is considered a drought-resistant crop due to its efficient stress avoidance and stress-tolerance mechanisms,[8,9] which permit it to thrive in arid and semiarid areas, even under desert conditions.[10] Nevertheless, despite its toughness, pomegranate grown for commercial production in these conditions requires regular irrigation throughout the season to reach optimal growth, a marketable yield, and acceptable fruit quality,[11,12] and to reduce the incidence of fruit physiopathies[13,14] (e.g., fruit cracking and fruit splitting).

To cover fully the demands of modern consumers, in addition to pomegranates' health-related properties, the fruits need to be pesticide-free and excel in terms of attractiveness, mainly regarding size, skin redness, and the absence of physiopathies (e.g. sunburning, fruit cracking, fruit splitting or internal breakdown), insect attack injuries, or mechanical damage, while maintaining a pleasant taste. To this end, a fruit physical protection technique (preharvest fruit bagging) is sometimes used, alongside other

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farming practices, to improve fruit quality and to protect fruit from pathogens and pests, reducing the presence of pesticide residues. Indeed, this technique is very frequently used in peach, apple, pear, grape, and loquat farming in countries such as Japan, Australia, China, and the USA.[15,16] Nevertheless, the literature on the beneficial effects of fruit bagging is not unanimous and some contradictory results have been obtained.[16–18]

To the best of our knowledge, reports on the response of pomegranate fruit to bagging are very scarce, although Yuan *et al.*[19] showed that bagging is an effective cultivation measure to prevent pomegranate fruit cracking. Shlomo[18] recommended the use of bags open at the bottom as a good mechanical protection against important pest infestations. Meena *et al.*[20] demonstrated the potency of photoselective netting for improving the agroeconomic performance of pomegranate crops, especially in harsh climates and arid zones.

While pomegranate fruit quality parameters have been studied under different water deficit conditions,[9,13,21–23] the literature on the effect of bagging on fruit quality is very limited and there are no reports on the interaction of both factors applied simultaneously. The main objective of the current work was therefore to study the interaction between preharvest pomegranate fruit bagging and plant water status on the sensory and quality attributes of the fruit. In addition, the effect of both factors on fruit sunburn and fruit splitting was studied as a complementary objective.

MATERIAL AND METHODS

Plant material, experimental conditions, and treatments

The experiment was performed in the summer of 2017 in the CSIC Experimental Station near Santomera (Murcia, Spain) (38° 6′ N; 1° 2′ W). The plant material consisted of own-rooted 7-year old pomegranate plants (*P. granatum* (L.) cv. Mollar de Elche) spaced following a 3 m × 5 m pattern. The soil of the plot was stony (33%, w/w) and shallow, with a clay-loam texture.

Fully irrigated plants (treatment FI) were irrigated during the night to above crop water requirements – 115% evapotranspiration (ETo) – using a drip irrigation system with one lateral pipe per tree row and four emitters (each delivering 4 L h^{-1}) per plant. Irrigation in water stressed plants (treatment WS) was withheld for 60 days (from day of the year, DOY, 209 to 269), when evaporative demand is very high and water availability for irrigation is very scarce. To guarantee the recovery of WS plants, reirrigation was performed at the levels used in FI from DOY 269 to 286 (harvest time). From DOY 209 (the end of fruit thinning) to harvest, pomegranate fruits from both irrigation treatments were submitted to bagging with Pantone® 1205C colored bags (262 mm × 397 mm) open at the bottom, made from externally glossy single layer cellulosic paper (grade: 50 g m^{-2}, Bendtsen porosity: 373 mL min^{-1}) and stapled tightly around the fruit peduncle. All fruits from the treatment trees were bagged, and formed treatment B, while treatment NB consisted of fruits that were not bagged. For prophylactic purposes, plants were sprayed with fungicide (containing 80% sulfur) and insecticide (10% of 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether) a week before the bagging practice.

Pomegranate fruits from each treatment were manually harvested on DOY 286 (13 October), when commercial maturity was reached. Twenty fruits from each replicate were immediately transported in ventilated plastic pallet boxes to the laboratory (a 15 min trip) and stored under controlled conditions (5 °C and 90% relative humidity, RH) for less than a week, until analysis.

The experiment had completely randomized design, with four replications, each replication consisting of three adjacent tree rows, each with 11 trees. Measurements were taken on the inner plants of the central row of each replicate, which were very similar in appearance, while the other plants served as border plants.

Measurements

Weather, plant water status, yield, morphology, fruit splitting, sunburn and color

Using an automatic weather station (Adcon Telemetry Gmb. Vienna, Austria), placed near the experimental plot, the following parameters were measured: wind speed 2 m above the soil surface, rainfall, solar radiation, air temperature and air relative humidity. Daily values of crop reference ETo were calculated using the Penman – Monteith equation.[24] Mean daily air vapor pressure deficit (VPDm) was calculated according to Allen *et al.*[24]

Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in two fully expanded leaves from the south-facing side and the middle third of the canopy of four plants per treatment. To allow the water tension in the leaf to come to equilibrium with the water tension in the stem, leaves were enclosed in a small black plastic bag and covered with aluminum foil for at least 2 h before measurements, which were made using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA). Detached leaves were placed into the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber).[25]

At harvest, the incidence of each disorder in pomegranate fruit was determined by counting the number of healthy or specific disorder affected fruit – splitting (SPI) or sunburn (SUI) – in all fruits from each replicate. The mean fruit weight (FW) of marketable fruit yield (MY) was determined according to the weight and number of fruits per box in two randomly selected boxes per replicate.

Equatorial diameter (ED) and peel thickness (PT) were measured in each fruit with a digital caliper on 12 fruits per replicate. To measure PT, pomegranate fruits were cut in half and the measurements were performed on two opposite points in the equatorial zone. After PT measurement, each fruit was emptied by hand. The arils were weighted to calculate their weight and the ratio arils:

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whole fruit weights (AW, %), and they were used for further analyses.

Pomegranate peel color was assessed at four equidistant points of the equatorial region of individual fruit (the same 12 used for the measurement of ED and PT) using a Minolta CR 2000 colorimeter (Osaka Japan). The arils obtained to calculate aril weight in each fruit were extended on a white plate and their color was assessed in ten different places on the plate, expressing the results using the CIE $L^*a^*b^*$ system.[26] The mean values for lightness (L^*), green-red (a^*) and blue-yellow (b^*) coordinates for each fruit were calculated. The objective color was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($H^\circ = \arctan(b^*/a^*)$).

Fruit total soluble solids, acidity, maturity index and moisture content

Using six pomegranate fruits cut in half per replicate, juice was obtained by a squeezer (Braun model 3050, Barcelona, Spain). The total soluble solid (TSS) content of the juice was measured using a digital Atago refractometer (model N-20; Atago, Bellevue, WA, USA) and the titratable acidity was measured using acid-based potentiometry (877 Titrino plus; Metrohm ion analyses CH9101, Herisau, Switzerland). The maturity index was calculated as the ratio between the TSS and the titratable acidity.

Total phenolics content and total antioxidant activity of the fruit

The total phenol content of pomegranate aril juice was estimated using the Foling–Ciocalteu reagent following the recommendations of Singleton *et al.*[27] Absorption was measured at 760 nm using a UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves, with a concentration range between 0 and 0.25 g GAE L^{-1}, were used for the quantification of TPC, and showed good linearity ($r^2 \geq 0.996$).

According to Nuncio-Jáuregui *et al.*,[28] a methanol extract from each sample was prepared, to analyze the antioxidant activity (AA) by mixing 1 mL juice with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, before sonicating at 20 °C for 15 min and leaving for 24 h at 4 °C. Then the extract was sonicated again for 15 min, and centrifuged at 15 000×*g* for 10 min. The ABTS$^+$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation method was measured according to Re *et al.*[29] Briefly, 10 µL of the supernatant was mixed with 990 µL of ABTS$^+$ and, after allowing the reaction to proceed for 10 min, absorbance was measured at 734 µm. The absorbance was measured using a UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves in the range of 0.01–5.00 mmol Trolox L−1 were used for the quantification of antioxidant activity and showed good linearity (r2 ≥ 0.998).

Organic acids and sugars

Organic acids and sugars were quantified according to Melgarejo-Sánchez *et al.*[30] For this, 20 mL of pomegranate juice, obtained by squeezing the arils, was centrifuged at 15 000×*g* for 20 min (Sigma 3-18K, Osterode and Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm cellulose nitrate membrane filter and the samples (10 µL) were injected onto a heated (30 °C) Supelcogel™ C-610H column (30 cm × 7.8 mm i.d., Supelco, Bellefonte, PA, USA) protected with a Supelcogel C610H guard column (5 cm × 4.6 mm, Supelco, Inc.). The HPLC system used was a Hewlett-Packard 1100 series model (Wilmington DE, USA) with an autosampler and an UV detector, set at 210 nm, coupled to a refractive index detector (HP 1100, G1362A). The elution system consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Standard curves of pure organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, succinic, and fumaric acids) and sugars (glucose, fructose and sucrose) were used for quantification. Calibration curves, with a concentration range between 1 and 10 g L^{-1}, were used for the quantification of organic acids and sugars, and showed good linearity (r^2 0.999). Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

Fuit sensory analysis

Eleven trained panelists (four men and seven women aged between 24 and 70 years), all with lengthy experience in testing pomegranates, described pomegranate arils based on their expertise and training. The samples (codified using three-digit numbers) were served on odor free disposable plastic plates at room temperature, and water was provided to clean panelists' palates between samples. The panelists evaluated samples according to the lexicon reported by Vázquez-Araújo *et al.*[31] using a numerical scale to quantify the intensity of the attributes of the arils, where 0 represents no intensity and 10 extremely high intensity, with 0.5 increments.

Statistical analysis

Data were analyzed using SPSS software.[32] Two-way analysis of variance was performed considering two independent variables or factors (irrigation and bagging), each one having two different levels (FI and WS for irrigation factor and B and NB for bagging factor). Mean values were compared by Tukey's multiple range test at $P < 0.05$. AW, SPI and SUI percentage values were arc-sin-transformed before statistical analysis because they were not normally distributed. Ψ_{stem} values for each replicate were averaged before the mean and the standard error of each treatment was calculated.

RESULTS

During the experiment, the meteorological characteristics were those of a classic Mediterranean climate. Average daily maximum and minimum air temperatures were 30 and 18 °C, respectively. The VPDm ranged from 0.23 to 2.06 kPa, the ETo amounted to 313 mm and the rainfall was 51 mm, which fell mainly on DOY 241, 242, 245, and 250 (21, 7, 18 and 4 mm, respectively) (Fig. 1).

Throughout the experimental period, there were no differences in Ψ_{stem} values between B and NB plants under FI and WS conditions (data not shown). However, the Ψ_{stem} values in FI and WS plants showed significant differences (Fig. 2). Ψ_{stem} values in FI plants remained high and near stable during this period, showing mean values of −0.99 MPa

. By contrast, during the water withholding period, Ψ_{stem} values in WS plants gradually fell, to reach minimum values of −2.40 MPa on DOY 230. Rainfall increased Ψ_{stem} values in WS plants up to values similar to those in FI plants on DOY 244, and after this Ψ_{stem} values in WS plants once again decreased gradually as a result of the water withholding effect, reaching minimum values of −2.20 MPa before irrigation was resumed (DOY 269) (Fig. 2). Moreover, when irrigation was resumed, a very rapid recovery of Ψ_{stem} values in WS plants was observed (Fig. 2).

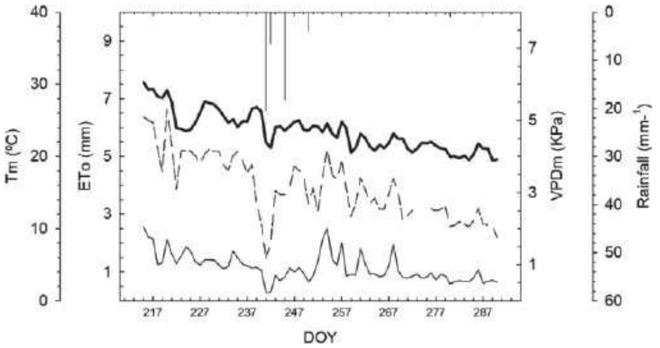

Figure 1. Daily crop reference evapotranspiration (ETo, dashed line), daily mean air temperature (Tm, solid bold line), mean daily air vapor pressure deficit (VPDm, solid thin line) and daily rainfall (vertical bars) during the experimental period (DOY 209−286).

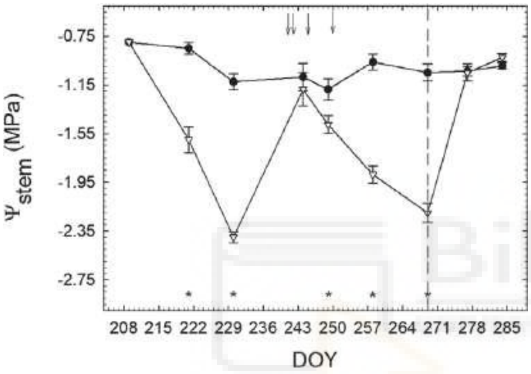

Figure 2. Midday stem water potential (Ψ_{stem}) values for pomegranate trees in FI (closed circles) and WS (open triangles) treatments during the experimental period. Vertical bars on data points are ± standard error of the mean (not shown when smaller than the symbols). Asterisks indicate significant differences between treatments. Vertical dashed line indicates the end of the period for which irrigation was withheld. Arrows indicate daily rainfall events.

Withholding irrigation water induced a decrease in fruit size and a substantial increase in the number of pomegranate fruits affected by fruit splitting. Consequently, FI and WS plants showed greater differences in marketable yield than in total yield (Table 1). Marketable yield in the B treatment plants was significantly higher than in the NB treatment plants due to the high incidence of sunburn in NB fruits. In addition, pomegranate B fruits were significantly smaller and had a significantly thinner peel than NB fruits (Table 1). Considering the interaction between irrigation and bagging, it is important to note that the bagging effect on marketable yield was significant only under WS conditions, whereas the bagging effect on fruit weight, fruit equatorial diameter, and peel thickness was significant only under FI conditions. Moreover, despite the absence of any effect of irrigation or bagging on arilsweight, the interaction of both factors showed that aril weight values in B fruits were significantly higher than in NB fruits, but only under FI conditions (Table 1).

Water withholding and bagging affected some chromatic characteristics of the pomegranate fruit peel. For example, *L**, *b**, *C** and *H*° values were significantly lower (darker and with a more intense blue color) in WS than in FI fruit peel, whereas bagging increased *b** and *C** values (more objective yellow color) compared to those in NB fruit peels. However, *a** values were not affected by the water deficit effect or by bagging (Table 2). As regards the interaction between the two factors considered here, the effect of irrigation on *L** values was significant only when FIB and WSB fruits were considered (irrigation was more important than bagging). The *b** and *C** values in FIB fruits were also higher (more objective yellow color) than in WSNB but similar to those in FINB and WSB fruits, and *H*° values in FIB fruits were significantly higher (more yellowish in color) than those in WSB and WSNB but similar to those in FINB fruits (Table 2). Conversely, the effect of both treatments (bagging and irrigation) on pomegranate aril color was different from that observed in pomegranate peel (Table 3). In this sense, FI arils showed higher *b** and *C** values (more objective yellow color) than WS arils, as was also observed in pomegranate peel, but this did not affect *L** and *H*° values, whereas B arils had significantly higher *b** and *H*° values (more yellowish and less reddish color) than NB arils although the *C** values remained constant. Like pomegranate peel, differences in *b** values between treatments were significant only when FIB and WSNB fruit were considered (Table 3).

The succinic acid, glucose, and fructose content of pomegranate fruit was not affected by bagging or by water withholding effect (Table 4). However, bagging decreased the citric acid and TSS content significantly and increased the titratable acidity content significantly, which led to a significant decrease in maturity index values. By contrast, WS increased TSS, leading to significantly higher maturity index values (Table 4). Regarding the interaction between bagging and water withholding, the bagging effect on titratable acidity and TSS was significant only in FI and WS plants, respectively, whereas the bagging effect on maturity index values was significant only in FI plants. The bagging

Table 1. Effects of different irrigation and bagging treatments on pomegranate total yield (TY, kg tree-1), marketable yield (MY, kg tree-1), average fruit weight (FW, g), fruit equatorial diameter (ED, mm) peel thinckness (PT, mm), arils weight ratio (AW, %) and fruit physiopathies incidence (splitting (SPI, %) and sunburn (SUI, %)). B = bagged fruits, FI = full irrigation, NB = not-bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant.

Treatment	TY	MY	FW	ED	PT	AW	SPI	SUI
ANOVA								
Irrigation	***	***	***	***	n.s.	n.s.	***	n.s.
Bagging	n.s.	**	***	***	***	n.s.	n.s.	***
Irrigation × bagging	**	***	***	***	***	**	**	***
Irrigation								
FI	63.38a	50.83a	454.83a	95.80a	5.29a	52.95a	7.22b	12.23a
WS	39.70b	26.53b	429.87b	93.76b	5.41a	53.96a	20.82a	12.74a
Bagging								
B	50.79a	42.19a	399.07b	92.70b	4.98b	53.80a	14.48a	3.57b
NB	52.29a	35.18b	485.63a	96.86a	5.72a	53.11a	13.55a	21.40a
Tukey's multiple range test								
Irrigation × bagging								
FIB	60.30a	48.68a	396.85b	92.50b	4.51b	55.48a	7.79b	5.49b
FINB	66.46a	52.99a	512.80a	99.09a	6.07a	50.42b	6.64b	18.97a
WSB	41.29b	31.39b	401.28b	92.90b	5.45a	52.11ab	22.32a	1.65b
WSNB	38.12b	21.68c	458.46ab	94.62b	5.36a	55.80a	19.31a	23.82a

Table 2. Effects of different irrigation and bagging treatments on pomegranate peel lightness (CIE L^*), red/greenness (CIE a^*), yellow/blueness (CIE b^*), chroma (C^*), and hue angle (H°) values. B = bagged fruits, FI = full irrigation, NB = not-bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	L^*	a^*	b^*	C^*	H°
ANOVA					
Irrigation	***	n.s.	***	***	***
Bagging	n.s.	n.s.	***	***	n.s.
Irrigation × bagging	*	n.s.	***	**	*
Irrigation					
FI	60.71a	22.73a	30.88a	39.68a	54.60a
WS	58.09b	23.58a	29.34b	38.66b	52.01b
Bagging					
B	59.39a	23.13a	30.91a	39.98a	54.35a
NB	59.41a	23.18a	29.31b	38.36b	52.25a
Tukey's multiple range test					
Irrigation × bagging					
FIB	61.09a	21.98a	31.83a	40.28a	56.79a
FINB	60.33ab	23.48a	29.94ab	39.07ab	52.40ab
WSB	57.70b	24.29a	30.00ab	39.67ab	51.91b
WSNB	58.49ab	22.87a	28.68b	37.64b	52.11b

effect on the citric acid content was significant only in WS plants (Table 4).

Neither bagging induced significantly higher AA-ABTS values than NB fruits (Table 4). The WS effect on AA-ABTS values was significant only in bagged plants (FIB and WSB), while B only affected FI fruits (Table 4).

Only three of the 33 sensory attributes of pomegranate fruits evaluated were affected by WS, whereas bagging did not affect any sensory attribute level (Fig. 3). Specifically, apple, pomegranate, and fruity flavors significantly increased in WS fruits; in this way, the treatments WSNB (red line) and WSB (yellow line) were the treatments having significantly ($P < 0.05$) higher intensities of key sensory attributes (pomegranate and fruity flavors) for the quality of pomegranate arils.

Table 3. Effect of different irrigation and bagging treatments on pomegranate aril lightness (CIE L^*), red/greenness (CIE a*), yellow/blueness (CIE b^*), chroma (C^*), and hue angle (H°) values. B = bagged fruits, FI = full irrigation, NB = no bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	L^*	a^*	b^*	C^*	H°
ANOVA					
Irrigation	n.s.	n.s.	***	***	n.s.
Bagging	n.s.	n.s.	***	n.s.	***
Irrigation × bagging	n.s.	n.s.	**	n.s.	n.s.
Irrigation					
FI	33.32a	19.51a	9.55a	21.93a	26.26a
WS	33.85a	17.79a	8.43b	19.88b	25.59a
Bagging					
B	33.96a	18.90a	9.69a	21.48a	27.49a
NB	33.22a	18.40a	8.29b	20.33a	24.35b
Tukey's multiple range test					
Irrigation × bagging					
FIB	34.69a	19.96a	10.56a	22.89a	28.47a
FINB	31.95a	19.06a	8.54ab	20.97a	24.03a
WSB	33.22a	17.83a	8.81ab	20.07a	26.50a
WSNB	34.48a	17.74a	8.04b	19.69a	24.66a

Table 4. Effect of different irrigation and bagging treatments on pomegranate fruit total soluble solids (TSS, °Brix), titrable acidity (TA, g anhydrous citric acid L^{-1}), maturity index (MI, TSS/TA), citric acid (CA, g 100 mL^{-1}), succinic acid (SA, g 100 mL^{-1}), glucose (Glu, g 100 mL^{-1}), fructose (Fru, g 100 mL^{-1}), total polyphenols content (TPC, mg GAE 100 g^{-1}), and total antioxidant activity measured according to ABTS$^+$ assay (AA-ABTS$^+$, mmol Trolox kg^{-1} dw) content. B = bagged fruits, FI = full irrigation, NB = no bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	TSS	TA	MI	CA	SA	Glu	Fru	TPC	AA-ABTS$^+$
ANOVA									
Irrigation	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	***
Bagging	***	***	***	***	n.s.	n.s.	n.s.	n.s.	***
Irrigation × bagging	***	**	***	***	n.s.	n.s.	n.s.	n.s.	***
Irrigation									
FI	15.51b	2.51a	62.15b	0.40a	0.29a	2.33a	2.83a	460.73a	19.20a
WS	17.19a	2.60a	66.26a	0.31a	0.20a	2.66a	3.21a	485.80a	14.12b
Bagging									
B	16.05b	2.64a	61.04b	0.26b	0.26a	2.96a	3.53a	463.82a	18.89a
NB	16.66a	2.47b	67.37a	0.45a	0.24a	2.04a	2.50a	428.70a	14.44b
Tukey's multiple range test									
Irrigation × bagging									
FIB	15.51c	2.71a	57.59b	0.26ab	0.26a	3.52a	4.27a	487.58a	24.60a
FINB	15.52c	2.32b	66.71a	0.50a	0.32a	1.63a	1.96a	433.89a	13.81b
WSB	16.58b	2.58ab	64.50a	0.16b	0.18a	2.34a	2.82a	440.00a	13.19b
WSNB	17.80a	2.63ab	68.03a	0.46a	0.22a	2.99a	3.60a	531.60a	15.05ab

DISCUSSION

The high and near constant Ψ_{stem} values in the FI plants (Fig. 2) suggest that their water requirements were covered.[13] The very low minimum Ψ_{stem} values before and after the rainfall episodes (DOY 241−250) indicated that WS plants reached a severe WS level[13] (Figs 1 and 2), although these minimum Ψ_{stem} values were reached ata low rate of around 0.08 MPa day^{-1} and 0.04 MPa day^{-1}, before and after the rainfall episodes, respectively.[13]

In addition to the very high fruit splitting incidence in WS fruits, the marketable yield was lower than in FI plants due to the lower WS fruit size (Table 1). In this sense, it is known that water deficit during pomegranate fruit growth and fruit ripening affects yield and fruit size.[9,21,33,34] For this, these two phenological periods are considered as critical from the total yield point of view.

The decrease in peel thickness values in FI fruits as a result of bagging (FIB) could be due to the low light intensity and high humidity inside the bag, which can affect the cell structure and peel thickness.[17,35] However, despite this effect on peel thickness, and in contrast to the results obtained by

Yuan *et al.*,[19] fruit bagging did not affect fruit splitting in FI plants or in WS plants. This behavior could be attributed to the fruit peel characteristics of each cultivar, such as peel elasticity or tannic contents in the peel, which can lead to different behaviors under bagging conditions.[19] Whatever the case, this changing behavior suggested that the asymmetric rehydration of previously water stressed fruits is the main factor leading to pomegranate fruit splitting.[13]

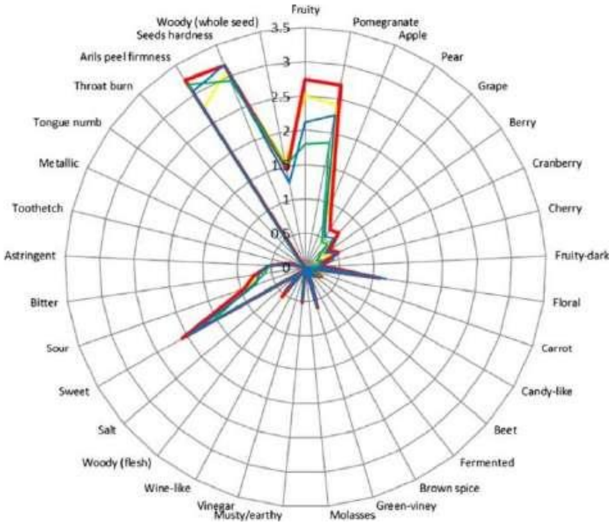

Figure 3. Descriptive sensory analysis in fully irrigated and not bagged (FINB, blue line), fully irrigated and bagged (FIB, green line), water stressed and not bagged (WSNB, red line) and water stressed and bagged (WSB, yellow line) pomegranate fruits.

The higher marketable yield in WSB than in WSNB was due to the high incidence of unmarketable fruits in WSNB plants caused by peel sunburn (Table 1). This physiological disorder arises from the fruit peel being directly exposed to high sunlight, which burns the fruit surface, decreasing its appeal and leading to unmarketable fruits, with inevitable economic losses.[36,37] Rabinowitch *et al.*[38] indicated that peel sunburn seems to be caused mainly by the concurrence of two factors, heat and light, the threshold values of which are cultivar dependent. However, the amount of time that is necessary to induce sunburn has not been established. Yazici and

Kaynak[39] showed that during July, August, and September, when air temperature and solar radiation are higher than 30 °C and 600 W m^{-2}, respectively, sunburn peel damage takes place. In this sense, it is important to emphasize that under our experimental conditions, daily maximum radiation values were above 600 W m^{-2} every day and on 38 days the maximum daily air temperature was above 30 °C, which could explain the high sunburn incidence in NB fruits (Table 1).

From the statistical point of view, bagging induced a significant overall tendency for peel and arils to turn more yellow (Tables 2 and 3), which supports the idea that bagging affected fruit growth and ripening. However, it is important to consider that chromatic changes observed in peel and arils were of limited real significance because changes of less than two units do not cause perceptible visual differences.[40–42]

The decrease in TSS values due to bagging in WS plants (Table 4) would have been due to the partial protection of the bagged fruits, which would decrease fruit water loss and would have favored water accumulation within the fruit. Moreover, a higher fruit water content would favor the translocation of soluble solids.[43,44] Similar results were obtained by Meena *et al.*[20] in pomegranate fruits under colored shade nets, by Amarante *et al.*[17] in bagged pear fruits and by Seeley *et al.*[45] in shaded apple fruits. However, Bentley and Viveros,[46] described how TSS increased in bagged apple fruits in only one of the two years of the experimental period, and Hofman *et al.*[47] concluded that TSS values in mango fruits were not affected by bagging. In any case, the decrease in maturity index values by the bagging effect in FI fruits as a result of the maintenance of TSS and the increase in titratable acidity values (Table 4) can be considered an unfavorable aspect because the overall consumer perception of pomegranate fruit quality is related more to maturity index than to the soluble sugar content alone.[48]

The decrease in antioxidant activity of pomegranate juice caused by the WS effect (Table 4) could be explained by the fact that WS inhibits the biosynthesis of punicalagin,[22] which significantly contributes to the antioxidant activity of pomegranate juice.[49] The increase in antioxidant activity observed in FI fruits as a result of the bagging effect (Table 4) contrasted with the results of Meena *et al.*,[20] who indicated that pomegranate fruits cultivated in open conditions have higher antioxidant activity than those grown under shade net, and hypothesized that plants probably use the induction of antioxidants as a protective measure to avoid peel sunburn. Weerakkody *et al.*[50] showed that sunscreen did not affect the total polyphenols content or the antioxidant activity of pomegranate juice. These different responses of antioxidant activity to bagging may be related to the characteristics of the bags in question mainly light transmittance.[51]

Only significant differences were detected in apple, pomegranate, and fruity flavor notes as a result of WS, but no significant differences were observed as result of the bagging. This finding indicated that the chemical changes occurring in the pomegranate fruits (Table 4) were not reflected in significant changes in the organoleptic attributes (Fig. 3). However, it is also possible that the heterogeneity of the fruits can mask the small differences in the sensory attributes due to the bagging factor; this masking effect of fruit heterogeneity on quality parameters is quite frequent in fruits and vegetables. The increase in TSS and maturity index values as a result of WS and the decrease in the same values as a result of bagging were not related to changes in the corresponding sensory attributes (sweetness, pomegranate, apple, pear, fruity dark, grape, berry, cranberry, and floral notes).[31] Finding no significant effect of bagging on sensory qualities is a positive result and indicated that the inner quality of the pomegranates was as good as that of the control samples.

CONCLUSIONS

In summary, the observations discussed above suggested that preharvest pomegranate fruit bagging may have certain negative effects in terms of slowing down fruit growth and ripening. However, such negative aspects may be outweighed by other very important commercial benefits such as the increase in antioxidant activity and the reduction in peel sunburn. Moreover, the chemical changes that occurred in pomegranate fruits as a result of bagging and WS were not perceived by the trained panellists. The fruits' response to bagging interacts with plant water status and probably with other environmental or farming practices. This would explain why the literature on fruit responses to preharvest bagging is not unanimous and contradictory results are frequent. Future research into fruit bagging should take into consideration that, in addition to the specific characteristics (physical and chemical characteristics, size, color, basal aperture or not, etc.) of the bags, other factors and environmental conditions may interact and affect the fruit response to bagging. These factors would include the plant cultivar, the phenological period during which fruits are bagged, the material of the bags, the duration of fruit exposure to natural light from the time the bags are removed until fruit harvesting, and cultivation practices (irrigation, pruning and thinning characteristics, among others).

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7.7 Publication 7 (Literal Transcription)

Reducing incidence of peel physiopathies and increasing antioxidant activity in pomegranate fruit under different irrigation conditions by preharvest application of chitosan

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Reducing incidence of peel physiopathies and increasing antioxidant activity in pomegranate fruit under different irrigation conditions by preharvest application of chitosan

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A B S T R A C T

No previous information exits on the effect of preharvest chitosan, a plant biostimulant and antitranspirant, which has been considered as a food additive by the USFDA, spraying on pomegranate trees. Then, the effect of chitosan spraying in fully irrigated and water stressed trees on yield, fruit quality and the occurrence of fruit peel physiopathies was studied. Some of these effects were negative such as the reduction in fruit weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel cracking or splitting and fruit sunburn physiopathies occurrence, which would considerably improve the returns of pomegranate growers.

1. Introduction

Although pomegranate (*Punica granatum* L.) is frequently considered a crop of minor importance, it is one of the oldest known edible fruits. Mainly grown in semi-arid mild-temperate to subtropical climates (Blumenfeld et al., 2000), pomegranate confronts water deficit by developing stress avoidance and stress tolerance mechanisms (Rodríguez et al., 2012), which endow it with the capacity to support heat and to thrive in arid and semiarid areas, even under desert conditions (Aseri et al., 2008).

In recent years, pomegranate fruit consumption has been increasing due to its perceived health-related characteristics such as its antiatherosclerotic effects, which are able to reduce blood pressure (Aviram et al., 2008), its high antioxidant activity (Gil et al., 2000; Seeram et al., 2006) and the anticarcinogenic compounds it contains (Malik et al., 2005; Malik and Mukhtar, 2006; Adhami and Mukhtar, 2006). To fulfil consumer satisfaction, its health-related properties and pleasing taste need to be accompanied by an attractive appearance in terms of size and redness, and the absence of pesticide residues, insect attack injuries and mechanical damage.

Under Mediterranean culture conditions, the incidence of some pomegranate physiopathies, mainly sunburn, cracking and splitting, is frequent, making fruits unmarketable and causing substantial economic losses to farmers, who may lose half of their crop yield (Blumenfeld et al., 2000; Melgarejo et al., 2004; Yazici and Kaynak, 2009). Pomegranates are terminal-bearing plants with thin branches, which bend

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under the fruit's weight, and peel sunburn occurs mainly in fruits that previously developed in the shade (Melgarejo et al., 2004). For their part, fruit cracking and splitting are the result of changes in fruit water relations and fruit skin properties (Rodríguez et al., 2018). In fact, when previously stressed fruits are rehydrated, an asymmetric increase in turgor pressure takes place. So, aril turgor increases to a much greater extent than peel turgor. This increase in aril pressure puts pressure on the peel and make it susceptible to cracking and/or splitting, especially bearing in mind that, under water stress, the mechanical properties of peel change, peel elasticity tending to decline, so that the peel becomes thicker and stiffer (Galindo et al., 2014b; Rodríguez et al., 2018).

Chitin is a naturalpolysaccharide consisting of a copolymer of *N*-acetyl-$_{D}$-glucosamine and $_{D}$-glucosamine residues, linked by β-1,4 glycosidic bonds (Nge et al., 2006). It is found in various sources in nature such as the shells of crustaceans, cuticles of insects and the cell wall of fungi and some algae, where it is produced by demineralization and deproteinization (Rinaudo, 2006; Pichyangkura and Chadchawan, 2015; Younes and Rinaudo, 2015).

Chitosan is the deacetylated (water soluble) form of chitin and is the overall name of a group of heteropolysaccharides differing in structure, molecular weight, degree of acetylation and properties such as pKa, solubility, viscosity, etc. (Falcón-Rodríguez et al., 2012; Pichyangkura and Chadchawan, 2015). Chitosan is considered a biostimulant because it has broad applications in crop culture for its significant antimicrobial activity (Palma-Guerrero et al., 2008; Badawy and Rabea, 2012; Falcón-Rodríguez et al., 2012), and through (i) promoting the growth of roots, shoots and leaves of various plants (Chibu and Shibayama, 2001; Wanichpongpan et al., 2001), (ii) increasing crop yield (Katiyar et al., 2014), (iii) conserving water use due to its effective antitranspirant effect (Bittelli et al., 2001), (iv) mitigating the effects of water stress (Yang et al., 2009) and (v) decreasing postharvest table grape cracking (Zoffoli et al., 2008; Shiri et al., 2013).

Despite the above mentioned beneficial characteristics, there have been no reports on the effect of chitosan spray on pomegranate yield and fruit quality, or on the interaction of this factor and plant water status. Consequently, the main objective in the current study was to analyse the interaction between preharvest pomegranate fruit chitosan spraying and plant water status on yield and the occurrence of fruit cracking or splitting and fruit sunburn physiopathies. In addition, the effect of both factors and their interaction on fruit quality attributes was studied as a complementary objective.

2. Materials and methods

2.1. Plant material, experimental conditions and treatments

The experiment was carried out in the summer of 2017 in the Tres Caminos Experimental Station near the city of Santomera (Murcia, Spain) (38°6′ N; 1°2′ W). The plot soil was stony (33%, w/w) and shallow, with a clay-loam texture. The plant material consisted of own rooted 7-year old pomegranate trees (*P. granatum* (L.) cv. Mollar de Elche) in a 3 × 5 m spacing pattern.

Irrigation was performed daily and during the night using a drip irrigation system with one lateral pipe per tree row and four emitters (each delivering 4 L h^{-1}) per plant. Fully irrigated plants (FI) were irrigated above the estimated crop water requirements (115% crop reference evapotranspiration, ETo) while irrigation was withheld from the water stressed plants (WS) from the day of the year (DOY) 221 to DOY 269 (48 days), after which, irrigation was resumed at FI level until harvest (DOY 286, 13 October). In addition, on day of the year, DOY, 221 (10 days after the end of fruit thinning) and on DOY 254, plants from both irrigation treatments were sprayed with a Quitomax® solution at 45 g of active ingredient per ha. This active ingredient consists of chitosan polymers of medium molecular weight (≥ 100 kDa), obtained with basic deacetylation from chitin. Plants treated with Quitomax® comprised treatment Q, while treatment NQ consisted of plants that were not sprayed with chitosan. Pomegranate fruits from each replicate were manually harvested on DOY 286, when commercial maturity (colour and size sufficiently attractive for consumers) was reached and 18 fruits from each replicate were immediately transported under ventilated conditions to the laboratory and stored under controlled conditions (5 °C and 90% relative humidity, RH) for less than a week, until analysis.

The design of this experiment was completely randomized with four replications. Three adjacent eleven tree rows were used per each replication. The inner plants of the central row of each replicate were used for measurements, whereas the other plants served as border plants.

2.2. Measurements

2.2.1. Weather, plant water status, yield, fruit cracking or splitting and sunburn

Wind speed 2 m above the soil surface, rainfall, solar radiation, air temperature and air relative humidity data were collected from an automatic weather station placed near the experimental plot. Daily values of ETo were calculated using the Penman-Monteith equation (Allen et al., 1998) and mean daily air vapour pressure deficit (VPDm) was calculated according to Allen et al. (1998).

Stem water potential (Ψ_{stem}, MPa) was measured at midday (12 h solar time), using a pressure chamber (PMS 600-EXP, PMS

Instruments Company, Albany, USA), in two fully expanded leaves from the southfacing side and middle third of the tree of four plants per treatment, which were enclosed in a small black plastic bag and covered with aluminium foil for at least 2 h before the measurements.

In order to estimate the marketable yield (kg tree^{-1}), the total number of harvested fruits from each replicate were counted, along with the number of fruits rejected because of sunburn, cracking and/or splitting disorder, the mean fruit weight of the marketable fruit yield was determined according to the weight and number of healthy fruits per box in two randomly selected boxes per replicate.

2.2.2. *Morphological fruit characteristics, arils and peel colour*

A digital calliper was used to measure the equatorial diameter of the fruit (mm), which were then emptied and the arils were weighed with a precision balance in order to calculate the arils percentage content.

Pomegranate peel colour was estimated with a Minolta CR 2000 colorimeter (Osaka, Japan), measuring the colour at four equidistant points of the equatorial region of each individual fruit. Arils in each fruit were spread on a white plate and their colour was assessed in ten different places of the plate, expressing the results in the CIE$L^*a^*b^*$ system. The mean values for lightness (L^*), green-red (a^*), and blue-yellow (b^*) coordinates for each fruit were calculated. The objective colour was calculated as chromaticity, colour saturation or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle or tone ($H^o = \arctan(b^*/a^*)$) (Galindo et al., 2015).

2.2.3. *Fruit chemical characteristics*

Pomegranates were squeezed with a manual squeezer to obtain the juice in order to analyze different chemical parameters. An acid-based potentiometer (877 Titrino plus; Metrohm ion analyses CH9101, Herisau, Switzerland) was used to measure the titratable acidity (Galindo et al., 2015) and a digital refractometer Atago (model N-20; Atago, Bellevue, WA) to measure the total soluble solids (ºBrix). The maturity index was calculated as the ratio between both parameters.

Organic acids and sugars (citric acid (CA), succinic acid (SA), glucose (Glu) and fructose (Fru), g 100 m L−1) were quantified according to Melgarejo-Sánchez et al. (2015). For this, 20 mL of juice obtained by squeezing the arils was centrifuged at 15,000 × g for 20 min (Sigma 3–18 K, Osterode & Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm cellulose nitrate membrane filter and the samples (10 µL) were injected onto a heated (30 °C) Supelcogel TM C- 610H column (30 cm × 7.8 mm i.d., Supelco, Bellefonte, PA, USA) protected with a Supelcogel C610H guard column (5 cm × 4.6 mm, Supelco, Inc.). The HPLC system used was a Hewlett-Packard 1100 series model (Wilmington Del., USA) with autosampler and UV detector, set at 210 nm, coupled to a refractive index detector (HP 1100, G1362 A). The elution system consisted of 0.1% phosphoric acid at a flow rate of 0.5 mL/min. Standard curves of pure organic acids and sugars were used for the quantification. Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

The total phenol content (TPC, mg GAE 100 g−1) of pomegranate fruits was estimated using the Foling-Ciocalteu reagent following the recommendations of Singleton et al. (1999).

A methanol extract of each sample was prepared in order to analyze the antioxidant activity (AA) by mixing 1 mL juice with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, before sonicating at 20 °C for 15 min and leaving for 24 h at 4 °C. Then the extract was sonicated again for 15 min, and centrifuged at 15,000 x g for 10 min. The ABTS+ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation method was used according to Re et al. (1999). Briefly, 10 µL of the supernatant were mixed with 990 µL of ABTS+ and after allowing the reaction to proceed for 10 min, the absorbance was measured at 734 µm. The absorbance was measured by UV–vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). The calibration curves, in the range 0.01–5.00 mmol Trolox L−1 were used for the quantification of antioxidant activity (mmol Trolox kg−1 dw) and showed good linearity (r2 ≥ 0.998).

2.3. Statistical analysis

The statistical analysis was a two-way ANOVA considering two independent variables or factors (factor A: irrigation and factor B: chitosan application), each one having two differentlevels (FI and WS for irrigation factor and NQ and Q for chitosan factor) and the software utilized was SPSS (2012). Mean values were compared by Tukey's multiple range test at $p < 0.05$. Ψstem values for each replicate were averaged before the mean and the standard error of each treatment were calculated. AW, SPI and SUI percentage values were arc-sin-transformed before statistical analysis because they were not normally distributed.

3. Results

Throughout the experimental period, VPDm ranged from 0.23 to 2.01 kPa and ETo amounted to 244 mm. Moreover, average daily maximum and minimum air temperatures were 30 and 18 °C, respectively. Total rainfall amounted to 51 mm, which fell mainly on DOY 241 (21 mm), 242 (7 mm), 245 (18 mm) and 250 (4 mm) (Fig. 1).

Similar Ψ_{stem} values were found in treated (Q) and non-treated (NQ) plants under FI and WS conditions (data not shown). Nevertheless, Ψ_{stem} values in FI and WS plants behaved differently,

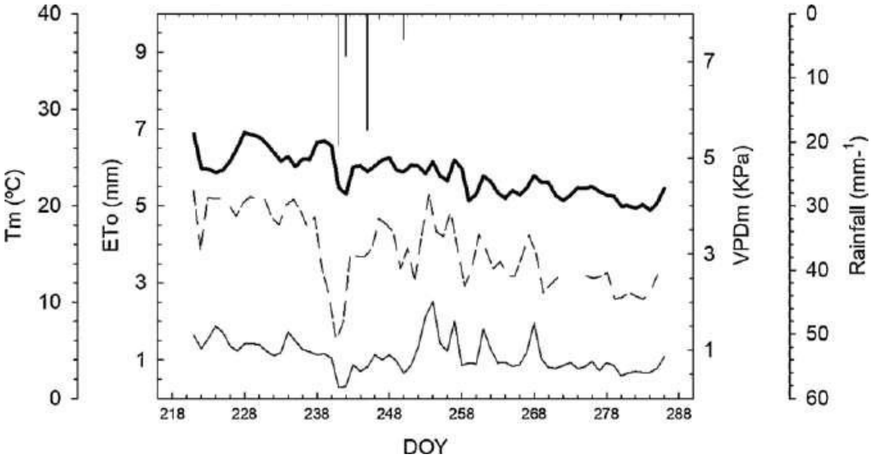

Fig. 1. Daily crop reference evapotranspiration (ETo, medium-medium line), daily mean air temperature (Tm, solid line), mean daily air vapour pressure deficit (VPDm, thin line) and daily rainfall (vertical bars) during the experimental period

remaining high and almost constant (average -1.02 MPa) in FI plants, and gradually decreasing in WS plants to reach minimum values of −1.60 MPa on DOY 230. These minimum Ψ_{stem} values in WS plants increased up to reach values similar to those of FI plants on DOY 249, when it rained. After this day, as a result of the water withholding effect, Ψ_{stem} values in WS plants decreased once again, reaching minimum values of -2.20 MPa at the end of the water withholding period (DOY 269). When irrigation was restarted in WS plants, their Ψ_{stem} value rapidly recovered (Fig. 2).

The total yield was lower in WS than in FI trees because of the smaller size of the WS fruit (Table 1). In addition, WS fruits showed a higher incidence of fruit cracking and/or splitting than FI fruits, which increased the difference between the marketable yield values of FI and WS (Table 1). Chitosan did not affect total yield, but induced smaller fruits. However, fruits from chitosan sprayed trees showed a lower incidence of sunburn and cracking and splitting, so that the marketable yield of treated plants was significantly higher than that observed in non-treated plants (Table 1).

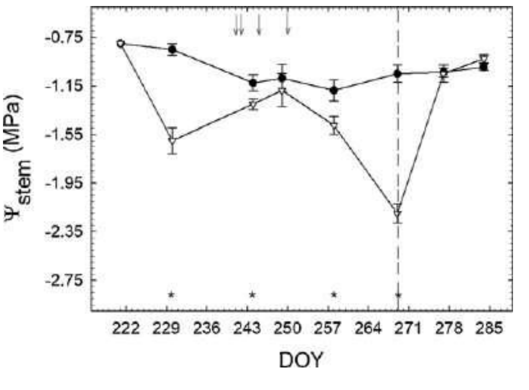

Fig. 2. Midday stem water potential (Ψ_{stem}) values for pomegranate trees in FI (closed circles) and WS (open triangles) treatments during the experimental period. Vertical bars on data points are ± s.e. of the mean (not shown when smaller than the symbols). Vertical medium-medium line indicates the end of the period for which irrigation was withheld. Arrows indicate daily rainfall events.

Table 1
Effect of irrigation and chitosan treatments on pomegranate total yield (TY, kg tree^{-1}), marketable yield (MY, kg tree^{-1}), average fruit weight (FW, g), fruit equatorial diameter (ED, mm), arils weight (AW, %), and fruit peel physiopathies incidence (cracking and/or splitting (SPI) and sunburn (SUI), %). FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*) or $P \leq 0.001$ (***). n.s. = not significant.

Treatment	TY	MY	FW	ED	AW	SPI	SUI
				ANOVA			
Irrigation	***	***	***	***	n.s.	***	n.s.
Chitosan	n.s.	***	***	n.s.	n.s.	***	*
Irrigation							
FI	60.26a	49.97a	506.52a	98.57a	52.49	3.17b	14.79
WS	43.52b	29.20b	437.38b	93.41b	51.91	15.12a	18.45
Chitosan							
NQ	51.90	35.43b	485.67a	96.85	52.18	11.63a	21.39a
Q	51.88	43.74a	453.66b	94.82	52.24	6.54b	10.25b
			Tukey`s multiple range test				
Irrigation x Chitosan	*.	***	***	***	n.s.	***	*
FINQ	58.33a	44.72b	512.88a	99.09a	50.43	4.36c	18.97a
FIQ	62.18a	55.22a	498.05b	97.86a	53.89	1.98c	9.21b
WSNQ	45.46b	26.13c	458.47ab	94.62ab	53.94	18.90a	23.82a
WSQ	41.57b	32.27c	409.26b	91.79b	50.58	11.09b	11.29b

As regards the interaction between irrigation and chitosan spraying, it is important to note that the chitosan spraying effect on fruit cracking and/or splitting incidence was only significant under WS conditions, whereas the effect on fruit sunburn was significant under both FI and WS conditions (Table 1). In this sense, the effect of chitosan spraying on marketable yield was only significant in FI trees.

The effects of irrigation water withholding and chitosan spraying on pomegranate peel colour were characterized by an increase in C* values and a decrease in b* and Hº values, respectively (Table 2). The interaction between both factors was significant only when peel b* and C* values were considered. In this sense, the effect of chitosan on b* values was significant only in FI fruits, while only peel C* values in fruits from the FINQ and WSQ treatments differed significantly, the FINQ values being significantly higher (Table 2).

Table 2
Effect of irrigation and chitosan treatments on pomegranate peel lightness (CIE *L**), red/greenness (CIE *a**), yellow/blueness (CIE *b**), chroma (*C**) and hue angle (*H*º) values. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at *P* ≤ 0.05 (*) or *P* ≤ 0.001 (***). n.s. = not significant.

Treatment	*L**	*a**	*b**	*C**	*H*º
			ANOVA		
Irrigation	n.s.	n.s.	n.s.	***	n.s.
Chitosan	n.s.	n.s.	***	n.s.	***
Irrigation					
FI	60.36	24.37	28.96	37.51b	51.50
WS	59.17	23.07	28.33	38.88a	50.53
Chitosan					
NQ	59.42	23.18	29.31a	38.36	52.26a
Q	60.23	24.44	27.75b	37.97	49.36b
			Tukey`s multiple range test		
Irrigation x Chitosan	n.s.	n.s.	*	*	n.s.
FINQ	60.33	23.48	29.94a	39.08a	52.41
FIQ	60.39	25.54	27.65b	38.62ab	48.03
WSNQ	58.49	22.87	28.68ab	37.65ab	52.11
WSQ	60.07	23.34	27.86b	37.32b	50.68

The effect of water withholding and chitosan spraying on pomegranate arils colour differed from that observed for pomegranate peel (Table 3). Irrigation water withholding did not affect aril the colour characteristics, whereas chitosan decreased a* and C* aril values and increased Hº aril values. The interaction between both factors showed that the chitosan effect on *L**, *a** and *H*º values was significant only in FI arils. In this sense, FIQ arils showed higher *L** and *H*º values, and lower *a** values than those in FINQ arils (Table 3). The effects of irrigation water withholding and chitosan spraying on the chemical characteristics of pomegranate fruit were very scarce (Table 4). The chitosan effect was significant only on the AA-ABTS$^{\cdot+}$ values, which increased in treated (Q) fruits. The effect of withholding irrigation water increased total soluble solids and titratable acidity and decreased the succinic acid content. In this respect, the interaction between these two factors was significant not only for total soluble solids, titratable acidity, succinic acid and antioxidant activity levels but also for the citric acid content (Table 4).The decrease in succinic acid through a water withholding effect was significant only when chitosan sprayed fruits were considered (FIQ and WSQ); chitosan induced a decrease in the citric acid content only in WSQ fruits and antioxidant activity increased through a chitosan effect in FIQ fruits (Table 4). Despite irrigation water withholding effect increased fruit titratable acidity, the interaction with chitosan spraying factor induced similar values in FIQ and WSNQ fruits, whereas these values in WSQ fruits were higher than those in FIQ and FINQ fruits. Even though the interaction between irrigation water withholding and chitosan spraying was significant, the chitosan effect was not significant in fruits from both plant water status considered (FI and WS) (Table 4).

Table 3
Effect of irrigation and chitosan treatments on pomegranate aril lightness (CIE *L**), red/greenness (CIE *a**). yellow/blueness (CIE *b**), chroma (*C**) and hue angle (*H*º) values. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at *P* ≤ 0.05 (*) or *P* ≤ 0.001 (***). n.s. = not significant.

Treatment	*L**	*a**	*b**	*C**	*H*º
			ANOVA test		
Irrigation	n.s.	n.s.	n.s.	n.s.	n.s.
Chitosan	n.s.	***	n.s.	***	***
Irrigation					
FI	34.60	17.37	8.88	19.01	27.61
WS	34.25	17.13	7.85	19.74	24.75
Chitosan					
NQ	33.22	18.40a	8.29	20.33a	24.35b
Q	36.02	15.71b	8.46	18.10b	28.64a
			Tukey`s multiple range test		
Irrigation x Chitosan	*	*	n.s.	n.s.	*
FINQ	31.95b	19.06a	8.54	20.98	24.03b
FIQ	38.12a	15.12b	9.33	18.09	32.39a
WSNQ	34.48ab	17.74ab	8.04	19.69	24.66b
WSQ	33.93ab	16.30ab	7.59	18.12	24.87b

4. Discussion

The high Ψ_{stem} values in FI plants throughout the experimental period (Fig. 2) suggested that control plants were under non-limiting soil water conditions (Galindo et al., 2013, 2014a). In contrast, at the at the end of the water withholding period, the low Ψ_{stem} values indicated that WS plants were experiencing a relatively strong water stress situation (Galindo et al., 2013) (Fig. 2), even though these minimum Ψ_{stem} values were not reached

Table 4
Effect of irrigation and chitosan treatments on pomegranate fruit total soluble solids (TSS, ºBrix). titratable acidity (TA, g citric acid L^{-1}). maturity index (MI, TSS/ TA), citric acid (CA, g 100 m L^{-1}), succinic acid (SA, g 100 m L^{-1}), glucose (Glu, g 100 m L^{-1}), fructose (Fru, g 100 m L^{-1}), total polyphenols content (TPC, mg GAE 100 g^{-1}) and total antioxidant activity measured according to ABTS^{+} assay (AA-ABTS^{+}, mmol Trolox kg^{-1} dw) content. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant.

Treatment	TSS	TA	MI	CA	SA	Glu	Fru	TPC	AA-ABTS
ANOVA test Irrigation	***	***	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.
Chitosan	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***
Irrigation									
FI	15.64b	2.35b	66.62	0.46	0.27a	3.20	2.52	425.9	17.84
WS	17.46a	2.63a	66.70	0.38	0.20b	2.01	3.85	461.1	18.07
Chitosan									
NQ	16.66	2.48	67.37	0.45	0.24	2.04	2.50	482.7	14.44b
Q	16.40	2.51	65.70	0.38	0.23	3.36	4.08	391.1	22.65a
Tukey`s multiple range									
Irrigation x Chitosan	***	*	n.s.	*	**	n.s.	n.s.	n.s.	*
FINQ	15.52b	2.33c	66.70	0.45a	0.25ab	1.09	1.41	433.8	13.82b
FIQ	15.78b	2.38bc	66.49	0.48a	0.29a	3.24	3.98	415.2	23.74a
WSNQ	17.80a	2.63ab	68.04	0.46a	0.22ab	2.99	3.60	531.6	15.05b
WSQ	17.01a	2.64a	64.91	0.27b	0.17b	3.48	4.19	367.1	21.56ab

progressively due to the rain that fell during the experimental period. The absence of a chitosan effect on Ψ_{stem} values in FI and WS at any time point examined (data not shown), agrees with the results of Yang et al. (2009), who concluded that spraying chitosan, at any of the concentrations tested, had no effect on apple predawn leaf water potential changes induced by drought.

As expected, WS fruits were smaller than those in FI plants because of pomegranate fruit growth and fruit ripening are critical phenological periods from the yield point of view since water deficit affects total yield and fruit size (Mellisho et al., 2012; Laribi et al., 2013; Galindo et al., 2017). Moreover, the fact that withholding irrigation water effect was larger on marketable yield than total yield was due to the high fruit cracking and/or splitting incidence in WS treatment fruits, which is directly linked to the fruit water status at the end of fruit growth and ripening phase. When previously water stressed pomegranate fruits are rehydrated, the increase in turgor pressure is higher in the arils than in the fruit peel, leading to fruit incidence of cracking and/or splitting physiopathies (Galindo et al., 2014b; Rodríguez et al., 2018).

It is well known that chitosan is an ideal fruit preservative coating due to its film-forming and physical and biochemical properties (Park et al., 2002; Romanazzi et al., 2002; Shiri et al., 2013). The plant response to exogenous chitosan application depends not only on its chemical characteristics and the concentration of the chitosan molecules (Lin et al., 2005; Limpanavech et al., 2008; Kananont et al., 2010) but also on the plant material (Ohta et al., 2004) and their developmental stage (Pornpienpakdee et al., 2010). Whatever the case, the semipermeable layer formed by polysaccharides like chitosan coating modifies the internal atmosphere of the fruit, and due to their hygroscopic properties enable the formation of a water barrier and consequently reduce external water transfer and decrease the rate of respiration, among other effects (Zhang and Quantick, 1997; Zhang et al., 2011). For these reasons, the reduction of fruit cracking and/or splitting incidence in WS fruits as a result of chitosan spraying may have been due to an effective antitranspirant effect of the chitosan coating, which led to a more conservative water use in treated fruits (WSQ) than in non-treated fruits (WSNQ) (Table 1). Hence, when irrigation was resumed, the increase in aril turgor pressure in WSQ fruits was lower than in WSNQ, so that the pressure of the arils on the peel (which favours cracking and/or splitting) was lower in WSQ fruits than in WSNQ fruits. On the other hand, the fact that sunburn incidence decreased in FIQ and WSQ fruits in relation to that observed in FINQ and WSNQ, respectively (Table 1), could be attributed to the characteristics of the chitosan film around the fruit, which would act as a physical barrier against overall heat stress, reflecting harmful UV and IR radiation away from plants and, consequently, preserving fruit peel from sunburn.

The effect of water withholding and chitosan spraying on pomegranate peel and arils colour were low (Tables 2 and 3). In this sense, the first factor did not affect arils colour, but induced an increase in peel brightness, whereas the second factor induced less reddish and duller arils and a less yellowish and more reddish peel. Furthermore, the interaction between chitosan spraying and water withholding treatments led to the peel from FIQ fruits being less yellow than in FINQ, while the peel in FINQ fruits was brighter than that of WSQ fruits (Table 2). This interaction also induced FI arils to be lighter and less red through a chitosan effect (Table 3). With regard to the effect of the first factor, in a comparison of different withholding irrigation water treatments during fruit growth and lateripening Galindo et al. (2017) described a significant effect of water stress on pomegranate peel colour because *L*, b* and H°* values of the peel tended to decrease with accumulated water stress effect. These different behaviours could be attributed to the fact that the response to water stress of fruit of a specific cultivar depends not only on the water stress level, but also of the phenological phase at which it takes place, its duration and its development rate (Galindo et al., 2018; Rodríguez et al., 2018). As regards the second factor, Munhuweyi et al. (2017) observed a significant effect of the pomegranate arils cultivar in response to chitosan treatment during postharvest cold storage, because Wonderful cv. arils colour did not change, whereas Herskawitz cv. arils showed a less red and yellower colour, especially at the beginning of the storage period.

The increase in total soluble solids as a result of irrigation water withholding is in line with the results presented by Laribi et al. (2013) and can be attributed to the active hydrolysis of starch to sugars, whereas the increase in titratable acidity could be due to an increase in the conversion of soluble sugars into organic acids (Munhuweyi et al., 2017). The increases in total soluble solids and titratable acidity in pomegranate fruit can be considered as a positive characteristic from a consumer acceptance point of view (Martínez-Romero et al., 2013).

The increase in AA-ABTS$^{\cdot+}$ values through a chitosan effect agrees with the observations of Candir et al. (2018), who showed an increase in antioxidant capacity for pomegranate fruits treated with chitosan during cold storage. Similarly, Zahran et al. (2015) described an increase in antioxidant activity for pomegranate arils treated with irradiated chitosan during cold storage. However, the fact that chitosan increased AA-ABTS$^{\cdot+}$ values and did not affect TPC levels (Table 4) is not in line with the results of Borochov-Neori et al. (2009), who indicated that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this respect, to fully understand the antioxidant capacity and bioactivity of pomegranate fruits treated with chitosan, it is important to take into consideration that antioxidant activity of pomegranate arils is due to anthocyanin content, ascorbic acid and phenolic acids, either or in combination, are responsible for antioxidant activity of pomegranate arils (Sarkhosh et al., 2009). So, complementary analysis of fatty acids (Alcaraz-Mármol et al., 2015), organic acids such as gallagic acid, ellagic acid and gallic acids (Kulkarni et al., 2004; Calín-Sánchez et al., 2013), punicalin and punicalagin (Kulkarni et al., 2004) should be conducted.

5. Conclusions

Overall, the above-mentioned observations suggest that preharvest pomegranate fruit spraying with chitosan had little effect on peel and aril colour and the chemical characteristics of the arils. Some of these effects were negative such as the reduction in fruit weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel physiopathies, which would considerably improve the returns of pomegranate growers. In addition, it is important to consider that chitosan spraying constitutes a suitable and reliable cultural practice because chitosan is considered a food additive by the USFDA (United States Food and Drug Administration) and it has passed all the toxicological tests to which it has been submitted (Hirano et al., 1990).

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8. Results and Discussion

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Persimmon and quince plant water relations under drought

In agreement with the first objective, it was necessary to evaluate the sensitivity of measured indicators of the plant water status to use in irrigation scheduling in persimmon trees and to clarify the stress avoidance and stress tolerance mechanisms developed in response to mild water stress.

Troughout the experimental period and based on the fact that (i) θ_v values in T0 treatment were slightly above field capacity, (ii) Ψ_{pd}, Ψ_{md} and Ψ_{stem} values were nearly constant and very high in relation to the values already reported for other authors for full irrigated persimmon plants (Badal et al., 2010; Buesa et al., 2013), and (iii) Ψ_{pd} values depend mainly on soil moisture levels (Elfving et al., 1972; Torrecillas et al., 1988; Sellin, 1996), we conclude that T0 plants were under non-limiting soil water conditions. Moreover, considering that the tree water relations under flooding conditions are characterized by a substantial decrease in leaf conductance and leaf water potential as a consequence of the effects of chemical signals from roots and an increase in the resistance to water flowing through the plant (Ruiz-Sánchez et al., 1997; Dell'Amico et al., 2001), the water relations of T0 plants indicated the absence of any waterlogging because leaf turgor was maintained (Ψ_{ppd} and $\Psi_{pmd} > 0$), and high and near constant values of Ψ_{pd}, Ψ_{md}, Ψ_{stem}, SF and g_{lmd} were observed.

Regarding the T1 treatment, the fact that minimum θ_v values were around 81 % of field capacity and minimum Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} values were around - 0.50 MPa, - 1.17 MPa, - 0.87 MPa and 99.66 mmol m^{-2} s^{-1}, respectively, indicated that T1 plants were under a mild degree of water deficit during the experimental period (Cruz et al., 2012; Rodríguez et al., 2012; Torrecillas et al., 2018). In addition, the rate of development of water stress in T1 plants was very low because the Ψ_{pd}, Ψ_{stem} and Ψ_{md} values decreased by only around 0.01, 0.02 and 0.01 MPa per day basis (Hale and Orcutt, 1987).

The progressive decrease in g_{lmd} in T1 plants, and the tendency to shorten the duration of maximum stomatal opening in its circadian rhythm as stress progressed indicated that stomata regulation is a key mechanism in controlling leaf water status because leaf turgor was maintained in T1 plants and persimmon plants did not develop any other stress tolerance mechanism such as elastic adjustment (ϵ decrease) or active osmotic adjustment (Ψ_{os} decrease) in our experimental conditions. The decrease in g_{lmd} values of woody crop leaves in response to water deficit has been reported as a stress avoidance mechanism in response to water deficit, which improves water use efficiency (Rieger and Duemmel, 1992; Girona et al., 1993).

The behaviour of ϵ, Ψ_{os}, Ψ_{tlp} and RWC_{tlp} values, which did not change as a result of water deficit in T1 plants, was similar to the results obtained by other authors (Sánchez-Blanco et al., 1991; Savé et al., 1995; Torrecillas et al., 1996) suggesting that the ϵ and Ψ_{os} affect the RWC_{tlp} and Ψ_{tlp} values, respectively. RWC_a values in persimmon plants (around 58 %) were similar to those found for grapes (51–63%) (Rodrigues et al., 1993), to the lower limit of the range found for *Pinus ponderosa* (57-81 %) (Hardegree, 1989). and to the higher limit found for pomegranate (42-58%) (Rodríguez et al., 2012) and almond (42-59%) (Torrecillas et al., 1996). On the other hand, persimmon RWC_a values were high compared with other tree species such as apricot (27-42%) (Torrecillas et al., 1999), peach (29-44%) (Mellisho et al., 2011), *Eucalyptus globulus* (14-27%) (Correia et al., 1989) and *Quercus alba* (26-31%) (Parker and Pallardi, 1987). High RWC_a values represent a xeromorphic characteristic (Cutler et al., 1977), and are a consequence of thicker cell walls or differences in cell wall structure (Hellkvist et al., 1974), which could contribute to the retention of water when water potential decreases (Torrecillas et al., 1996).

SF, Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} encompass different time scales, because the last four are point measurements, taken at predawn or at midday, and are considered as indicators of the minimum (Ψ_{pd}) and maximum (Ψ_{md}, Ψ_{stem} and g_{lmd}) daily plant water deficit, whereas SF is an integrative indicator, which reflects the continuous sap flow records on a diurnal basis. Despite these facts, the relationships between SF and Ψ_{pd}, Ψ_{md}, Ψ_{stem} and g_{lmd} were high and constant, indicating that SF can be used to indicate the water status of young persimmon trees.

Assuming that any comparison of the sensitivities of different plant-based water status indicators for diagnosing water deficit must consider the strength of each indicator in the context of its variability (Goldhamer and Fereres, 2001, Naor and Cohen, 2003), it can be observed that Ψ_{md} was the most suitable indicator for persimmon irrigation scheduling when short periods of time are considered, because it showed the highest signal:noise ratio during the first 4 or 8 days of the experimental period (DOY 218-222 and 218-226). However, as the interval of time considered grew (DOY 218-231, 218-237, 218-244 and 218- 251) SF SI sharply increased and SF noise was maintained, leading it to show the highest signal:noise ratio for these intervals of time. Moreover, taking into consideration that during the two first periods of time considered (DOY 218-222 and 218-226) the SF signal:noise ratio, despite being lower than that showed by Ψ_{md} was relatively high, it could be concluded that SF is a more suitable indicator than Ψ_{md} for irrigation scheduling because it can provide continuous and automated registers of the plant water status in real time, avoiding frequent trips to the field and a significant input of manpower since frequent Ψ_{md} readings are needed.

In this respect, Ortuño et al. (2004) indicated that in young trees continuously measured plant water status indicators were more immediate and sensitive than discretely measured indicators for detecting water stress. Also, other authors indicated that MDS and SF revealed significant differences between irrigation treatments even in the absence of differences in Ψ_{stem} (Goldhamer et al., 1999; Remorini and Massai, 2003). By contrast, in persimmon plants, Badal et al. (2010) assessed the feasibility of using MDS, Ψ_{stem}, g_{lmd} and fruit diameter variations and concluded that although MDS can be successfully used as continuous plant water stress indicator, Ψ_{stem} was the most sensitive plant water stress indicator.

The above results indicated that persimmon plants exposed to mild water stress are able to gradually develop stomata control (a stress avoidance mechanism). Also, under water stress the high relative apoplastic water content could contribute to the retention of water. So, both drought resistance characteristics could have contributed to the leaf turgor maintenance observed during the experimental period. In addition, the discrete and continuously recorded plant-based indicators showed different degrees of sensitivity for diagnosing persimmon tree water status. Overall, SF measurements made by the heat-pulse technique are the most suitable method for estimating persimmon water status, because it showed the highest signal:noise ratio in almost all intervals of time considered, while providing continuous and automated registers of the persimmon water status in real time.

In accordance with the second objective, to study the quince tree response to water deficit it was necessary to clarify the plant water relations of quince trees under field conditions of water stress and during recovery, in order to identify the strategy (isohydric or anisohydric) by which quince plants cope with water stress and to elucidate the resistance mechanisms involved (avoidance and tolerance) developed.

Considering that Ψ_{pd} values depend on soil moisture conditions (Elfving et al., 1972), the high Ψ_{pd} values in T0 plants during the experimental period, which changed minimally, reflected adequate irrigation practices. At the end of the stress period, T1 plants showed Ψ_{pd}, Ψ_{md} and Ψ_{stem} values of -1.27, -3.70 and -2.35 MPa, respectively, indicating severe water stress levels. This water deficit in T1 plants was developed at a relatively low rate because Ψ_{pd}, Ψ_{md} and Ψ_{stem} decreased by only around 0.02, 0.04 and 0.02 MPa d^{-1}, respectively (Hale and Orcutt, 1987).

Under water stress conditions, the large differences between Ψ_{pd} and Ψ_{md} values in T1 plants and the rapid recovery of Ψ_{pd}, Ψ_{md} and Ψ_{stem} values when they were rewatered has also been observed in other crops subjected to severe water stress (Torrecillas et al., 1996; Ruiz-Sánchez et al., 1997; Rodríguez et al., 2012) and may be related with a high hydraulic conductivity, probably as a result of the resistance to cavitation observed in anisohydric plants (Ewers et al., 2005; Alsina et al., 2007).

In T1 plants, the gradual reduction in g_{lmd} values from the beginning of the stress period can be considered as a primary response to irrigation water withholding, which improves water use efficiency (Rieger and Duemmel, 1992; Rodríguez et al., 2012). In addition, the rapid recovery of g_{lmd} values in T1 plants when irrigation was resumed, suggests that stomatal aperture was controlled directly only by a hydroactive mechanism (Torrecillas et al., 1995). Then, as Rodríguez et al. (2012) observed in pomegranate leaves, to trigger hormonal changes in leaves able to delay stomatal aperture after rehydration (Davies and Zhang, 1991; Ruiz-Sánchez et al., 1997), it would have been necessary to reach a more severe water stress level.

Whatever the case, the low level of stomatal regulation, even when minimum soil water potential levels were achieved, led to pronounced decreases in Ψ_{md} values in T1 plants. the Ψ_{md} values in T0 and T1 plants correlated with Ψ_{pd} values, showing a slope higher than unity ($\sigma = 1.62$). For this, and in agreement with Martínez-Vilalta et al. (2014), quince plants can be categorized as being extremely anisohydric, which improves the drought resistance of the crop (Sade et al., 2012). Regarding this, in addition to the above mentioned advantage of being more resistant to cavitation, which permits anisohydric plants to recover rapidly following exposure to water stress, the low rate of stomatal regulation in such plants facilitates higher rates of leaf gas exchange than in isohydric plants (Franks et al., 2007).

Whatever the case, it is important to underline that very few species conform strictly to definitions of isohydric or anisohydric plants because plant species are ordered on a continuum rather than a dichotomy (Klein, 2014; Martínez-Vilalta et al., 2014). Therefore, plant species can move in this continuum through the effect not only of their physiological characteristics but also of the environment in which they grow and the conditions to which they are exposed at any given moment (Schultz, 2003; Domec and Johnson, 2012 ; Rogiers et al., 2012). Because of this, some plants can shift from being anisohydric to isohydric, depending on Ψ_{soil} (Domec and Johnson,2012).

The decrease in Ψ_{os} values in T1 plants (0.22 MPa) as a result of irrigation water withholding indicated the active accumulation of osmolytes and, consequently, active osmotic adjustments in the leaves. This behaviour agrees with those observed in other woody crops such as apple (Wang et al., 1995), apricot (Torrecillas et al., 1999), cherry (Ranney and Bassuk, 1991), jujube (Cruz et al., 2012), peach (Steinberger al., 1989; Arndt et al., 2000) and pomegranate (Rodríguez et al., 2012). In this sense, it is known that osmoregulation takes place mainly when water stress develops gradually over a prolonged period (Arndt et al., 2000), as in our experimental conditions, and it varies depending on the species and cultivar (Lakso, 1990; Torrecillas, 1996).

The maintenance of turgor in T1 plants even at maximum water deficit levels, when g_{lmd} values were still considerable, suggested that active osmoregulation contributed to maintaining quince leaf turgor (Ψ_{ppd} and Ψ_{pmd} above zero). Despite the fact that the level of osmotic adjustment achieved was able to contribute to leaf turgor maintenance, it was not able to modify the Ψ_{tlp} values. Other authors observed no changes in Ψ_{tlp} values despite the fact that these woody crops developed osmoregulation (Mellisho et al., 2011; Cruz et al., 2012; Rodríguez et al., 2012).

Contrary to the behaviour observed in some other woody crops (Savé et al., 1995; Sharon and Bravdo, 2001), quince plants were not able to develop elastic adjustment (Є decrease) in T1 plants due to the effect of a water deficit. Also, the similar behaviours observed in Є and RWC_{tlp} values agree with the results of Savé et al. (1995), who suggested that Є controlled RWC_{tlp}. Whatever the case, when plants are rehydrated after a water stress period, the maintenance of or an increase in cell wall rigidity may be necessary to maintain cell tissue integrity in species that show osmotic adjustment (Clifford et al., 1998; Álvarez et al. 2009).

The RWC_a values in quince plants, around 18-19 %, were close to those found in other tree species, such as *Eucalyptus globulus* (14–27%) (Hale and Orcutt, 1987; Correia et al., 1989) and *Quercus alba* (Parker and Pallardi, 1987), but lower than those found in other woody crops such as apricot (27-42%) (Torrecillas et al., 1999), peach (29-44%) (Mellisho et al., 2011), jujube (27-42 %) (Cruz et al., 2012), pomegranate (42-58%) (Rodríguez et al., 2012), almond (42-59%) (Torrecillas et al., 1996) and grapes (51-63%) (Rodrigues et al., 1993). These low RWC_a values are a consequence of thinner cell walls or differences in cell wall structure (Hellkvist et al., 1974). In contrast with that observed in some woody crops (Cruz et al., 2012), the consistency of the low quince leaf apoplastic water fraction under water stress prevents the accumulation of water in the apoplasm, leading to lower leaf water potential values and, as a consequence, a steeper gradient in water potential between the leaf and the soil, thus favouring water absorption.

The above results indicated that under our experimental conditions quince trees were characterized by an extreme anisohydric behaviour. During the development of the severe water stress and recovery periods, the plants suggested high hydraulic conductivity, probably because of the resistance to cavitation. In addition, the leaf conductance values observed suggested that stomatal aperture was controlled directly by a hydroactive mechanism alone. From the beginning of water stress to the time of maximum water stress, leaf turgor was maintained probably due to the contribution of the active osmotic adjustment (stress tolerance mechanism). This leaf turgor maintenance would allow substantial leaf conductance levels and, therefore, good leaf productivity. The constantly low quince leaf apoplastic water fraction under water stress could be considered as another drought tolerance characteristic, because, if the accumulation of water in the apoplasm is prevented, a steeper gradient in water potential between the leaf and the soil may occur during water stress, thus favouring water absorption.

Effect of water deficit during quince fruit growth and pomegranate fruit ripening on yield and fruit characteristics

In line with the third and the fourth partial objectives of this work, the effects of irrigation water withholding in the middle of the rapid quince fruit growth period and during the pomegranate ripening stage on fruit characteristics and composition were studied.

The fact that Ψ_{pd} values for control (T0) plants were high and near constant suggested that irrigation water requirements in control quince plants were adequately covered during the experimental period. In contrast, although the water stress in stressed (T1) plants developed slowly (0.019 MPa day^{-1}), the duration of the water withholding period (42 days) led to a substantial water stress level (Ψ_{pd} = -1.27 MPa). When T1 plants recovered, the Ψ_{pd} values rapidly increased (0.053 MPa day^{-1}), as has been observed in other fruit trees subjected to a strong water stress (Cruz et al., 2012; Mellisho et al., 2012; Galindo et al., 2016).

The water deficit achieved in T1 plants during the middle of the linear fruit growth phase indicated that this is a critical period for the quince marketable yield. This decrease in marketable yield (kg fruit/tree) was mainly due to decreased fruit size. Accepting that there is a direct relation between fruit turgor and growth, it is assumed that during the water stress period, T1 fruits lost turgor with a subsequent decrease in the fruit growth rate, which made it difficult for the full fruit size to be recovered when irrigation was restarted (Dell'Amico et al., 2012; Galindo et al., 2016, 2017; Rodríguez et al., 2018).

Several authors have observed that when the moisture content decreases, for example in jujube or plum fruits, fruit firmness increased as a consequence of a gummy and cork-like jujube flesh structure (Wu et al., 2012) or stiff plum flesh (Salvador et al., 2003). However, quince fruit firmness did not change despite the decrease in fruit moisture content, probably because this decrease in fruit moisture in T1 fruits was not strong enough to cause severe change or damage in quince flesh structure.

The high content of total polyphenolic content (TPC) in fruits from both irrigation treatments suggested that quinces, regardless of the fruit water status, can be used as an important source of natural antioxidants. This was even more interesting considering that quince fruits were harvested at commercial ripening (and can thus be considered as fully ripe), and that, according to Remorini et al. (2008), a decrease in TPC is a characteristic of the progress of ripening in fruits, and can be attributed to changes such as hydrolysis of glycosides, the oxidation of phenols by polyphenol oxidases and polymerization of free phenols.

The antioxidant capacity of quince flesh extracts was assessed using ABTS$^+$, DPPH$^\bullet$, and FRAP assays to evaluate the different modes of action of antioxidants (Prior and Cao, 1999; Huang et al., 2005). In this sense, the significant correlation between AA-ABTS$^+$ and TPC values indicated that TPC content determine the AA-ABTS$^+$ levels, whereas FRAP changes by water deficit effect were not directly mediated by TPC changes in this particular vegetal material. In addition, the constancy of AA-DPPH$^\bullet$ in response to water stress could be due to the fact that this test only considers lipophilic AA (Kuskoski et al., 2005), and is less sensitive than AA-ABTS$^+$ and AA-FRAP for measuring antioxidant defences (Redha et al., 2014).

In accordance with the quince fruit sensory analysis, the perceived increase in peel colour intensity (fruits were less green) in the middle of linear fruit growth phase could be used as a tool to improve T1 quinces agreed with the instrumental colour data, and, more precisely, with data on the green-red coordinate, *a** (Table 3). Regarding the texture attributes, it is important to underline that corkiness and fibreness in T1 quince fruits decreased by around 50% whereas crunchiness increased. Thus, water stress improved quince texture, because fruit corkiness and fibreness are two of the main handicaps for its consumption as fresh fruit (Szychowski et al., 2014).

According to the volatile profile of quince fruits, the high number of compounds identified confirmed the high odour and aroma intensity of these fruits. Water deficit induced important changes in an important number of these identified volatile compounds. These changes were characterized by decreases in the contents of ethyl octanoate and *E*,*E*-α-farnesene, which have pear, pineapple and apple sensory descriptors and increases in butyl acetate (banana), 1-hexanol (herbaceous) and ethyl hexanoate (apple and pineapple). An important fact is that even though the two most abundant compounds were found in T0, there were five compounds above 400 mg kg^{-1} dw in T1 quinces as compared to only 3 in T0 samples.

Bearing in mind the results from our pomegranate experiment and those from a previous paper (Galindo et al., 2014 a,b), it is clear that, in spite of the rainfall events (occurring on DOY 271), the cumulative water stress tended to increase with the number of days irrigation was withheld, the treatments in which irrigation was withheld during late fruit ripening (T1) and during the second half of fruit ripening (T2) producing a similar and moderate water stress level, and a more pronounced water stress level being observed in the treatments in which irrigation was withheld during ripening (T3) and at the end of fruit growth and ripening (T4). In addition, it is important to highlight the fact that the leaf conductance, stem, leaf and fruit water stress integral (SI_{gleaf}, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$, respectively) values showed some differences in describing the cumulative

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water deficit reached by the plants. $SI_{\Psi fruit}$ was the most reliable indicator to detect differences between the treatments at moderate water stress (T0 and T2), while SI_{gleaf} was the only indicator able to detect differences between the treatments at more pronounced water stress levels (T3 and T4).

The decrease in fruit yield in water stressed plants during the second half of fruit ripening (T2) and during fruit ripening (T3) confirmed the hypothesis that fruit ripening is a critical period from the yield point of view (Intrigliolo et al., 2013; Laribi et al., 2013). However, the fact that plants that were water stressed only at fruit late ripening stage (T1) showed similar marketable yield and fruit size to fully irrigated plants (T0) mean to clarify some aspects of the concept of phenological critical period (Goldhamer, 1989; Naor, 2006). In this sense, it is probable that sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Whatever the case, although pomegranate trees are able to withstand severe drought conditions (Rodríguez et al., 2012; Galindo et al., 2014a), irrigation was essential during most of the ripening stage to achieve optimum yield. According to Galindo et al. (2014a), the decrease in the marketable yield in T2, T3 and T4 plants was due mainly to the incidence of the fruit cracking and/or fruit splitting disorders and to the decrease in fruit size, which can be attributed to a loss of fruit turgor, because a direct relation between turgor and growth has been found in many studies (Serpe and Matthews, 2000; Matthews and Shackel, 2005).

In agreement with the results reported by Laribi et al. (2013) in pomegranate and Collado-González et al. (2014) in jujube fruits, withholding irrigation water during the ripening phase increases redness and darkness of the fruit peel. In this sense, the absence of data for peel pigments prevents any conclusion concerning whether the changes in peel colour were due to anthocyanin accumulation. Nevertheless, a negative correlation between lightness and pigment content is known, because as pigment levels increase, more light is absorbed, and lower values of luminosity are recorded. Moreover, considering that fruit peel from T1 plants, in which irrigation was withheld during late ripening for only 6 days, was also redder and darker than in fruits from T0, it is possible to rule out higher fruit exposure to sun-light as the only cause of colour changes (Gelly et al., 2004) because a significant reduction in the canopy characteristics is not very likely in only 6 days.

It is important to take remember that the first pomegranate fruits reaching the market fetch higher prices and, in this sense, 'Mollar de Elche' cultivar is often harvested when the peel has a sufficient red colouratiing (Manera et al., 2013). The significant increase in juice colour from T3 and T4 fruits is also very interesting for producers because pomegranate fruit attractiveness is primarily related to colour and taste parameters of the arils and their juice (Borochov-Neori et al., 2009). However, despite the fact that pomegranate colouration in pomegranates is predominantly due to anthocyanins (Shulman et al., 1984), TAC levels in T3 and T4 fruits were similar to that observed in T0 fruits. Laribi et al. (2013) showed also similar behaviour in juice from trees submitted to severe water restrictions during the last part of fruit growth and ripening period.

The fact that (i) TAC juice levels increased only in T1 fruits, (ii) TAA levels were similar in juices from the different irrigation treatments, and (iii) redness significantly increased only in T3 and T4 fruit juices, confirmed the view that juice antioxidant capacity is not linearly correlated with the red colour intensity, meaning that the anthocyanins are not major contributors to the antioxidant capacity exhibited by the pomegranates and their juice (Borochov-Neori et al., 2009). Moreover, the fact that withholding water irrigation decreased TPC levels and did not affect TAA levels does not agree with the linear relationship between soluble phenolic levels and antioxidant capacity indicated by Borochov-Neori et al. (2009), who supported the idea that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this sense, further analysis of fatty acids (Alcaraz-Mármol et al., 2015) and organic acids (Calín-Sánchez et al., 2013) must be conducted to fully understand the antioxidant capacity and bioactivity of pomegranate fruits subjected to deficit irrigation strategies.

The behaviour observed in TSS, TA, pH and MI juice values in response to irrigation withholding was not very clear and showed certain differences with respect to the results indicated by other authors in similar experiments. To be precise, Mellisho et al. (2012) indicated that arils from fruits exposed to water deficit during the second half of the linear fruit growth phase showed very similar overall chemical characteristics to arils from fully irrigated fruits, and Laribi et al. (2013) found a significant increase in TSS and TA levels in pomegranate juice from plants exposed to water deficit during the final phase of fruit growth and ripening. Additionally, when the effect of SDI applied throughout the season on pomegranate fruit quality is considered, the results at first sight are ambiguous. Mena et al. (2013) indicated that an SDI strategy that induced severe water stress led to pomegranate juice of lower visual attractiveness and less healthy (more yellowish, lower antioxidant activity and lower total phenolic compound, punicalagin and total anthocyanin contents) than the juice from fully irrigated trees. In contrast, Galindo et al. (2014b) concluded that SDI inducing severe water stress led to fruits with similar bioactive quality but a darker and more intense garnet colour than fully irrigated fruits, bringing the optimal harvest time by about 7–8 days. Also, Mellisho et al. (2012) showed that SDI inducing moderate water stress throughout the season led to changes in colour and chemical characteristics, which reflected earlier ripening.

In this sense, it is well known that water stress influences the content of secondary metabolites in plant tissues, having also contradictory results in other crops. For example, Chaves et al. (2007) reported the substantial accumulation of anthocyanins in grape berries under water stress. In contrast, Kennedy et al. (2000, 2002) showed that osmotic stress had little or no effect on anthocyanin accumulation in grape berries. This, at first sight, confusing relation between water stress and the production of bioactive compounds could be attributed to the fact that most manuscripts are not meticulous when it comes to recording aspects of plant water stress (precise phenological period at which it takes place, water stress rate of development, duration of maximum water stress, incidence of partial recoveries and other aspects) although such information is essential for the characterisation of experimental water stress conditions. In addition, it is essential to underline that is not possible to establish a linear correlation between water stress and secondary metabolite contents (Mattsson and Haack, 1987; Gobbo-Neto and Lopes, 2007). For this reason, Horner (1990) proposed a quadratic model to predict the concentration of phenolic compounds as a function of plant water status. So, under a mild water stress, CO_2 assimilation could be maintained and carbon-based secondary metabolites will probably increase when carbohydrates exceed the amount required for growth. Thus, mild osmotic stress may lead to a reduction in plant growth, accompanied by an increasing concentration of non-nitrogenous secondary metabolites. When water stress increases, stomatal regulation takes place and CO_2 assimilation is reduced. In this situation, carbon will be preferentially allocated to the synthesis of primary metabolites to the detriment of the synthesis of secondary metabolites (Mellisho et al., 2012).

Study of the effect of bagging and chitosan spraying on pomegranate fruit attributes and fruit peel physiopathies incidence

Taking into consideration the fifth and sixth partial objectives of this thesis, we studied the effect of the interaction between preharvest pomegranate fruit bagging and plant water status on the sensory and quality attributes of the fruit. In addition, the effect of both factors on fruit sunburn and fruit splitting was studied as a complementary objective. Also, we studied the interaction between preharvest pomegranate fruit chitosan spraying and plant water status on yield and the occurrence of fruit cracking or splitting and fruit sunburn physiopathies, and the effect of both factors and their interaction on fruit quality attributes.

Bearing in mind the results from our pomegranate bagging experiment, the high and near constant Ψ_{stem} values in the FI plants suggest that their water requirements were covered (Galindo et al., 2014b). The very low minimum Ψ_{stem} values before and after the rainfall episodes (DOY 241-250) indicated that WS plants reached a severe water stress level, although these minimum Ψ_{stem} values were reached at a low rate of around 0.08 MPa day^{-1} and 0.04 MPa day^{-1}, before and after the rainfall episodes, respectively.

In addition to the very high fruit-splitting incidence in WS fruits, the marketable yield was lower than in FI plants due to the lower WS fruit size. In this sense, it is known that water deficit during pomegranate fruit growth and fruit ripening affects yield and fruit size (Mellisho et al., 2012; Intrigliolo et al., 2013; Laribi et al., 2013; Galindo et al., 2017). For this, these two phenological periods are considered as critical from the total yield point of view.

The decrease in peel thickness values in FI fruits as a result of bagging (FIB) could be due to the low light intensity and high humidity inside the bag, which can affect the cell structure and peel thickness (Knoche et al., 2001; Amarante et al., 2002). However, in spite of this effect on peel thickness, and in contrast to the results obtained by Yuan et al. 2012), fruit bagging did not affect fruit-splitting in FI plants or in WS plants. This behaviour could be attributed to the fruit peel characteristics of each cultivar, such as peel elasticity or tannic contents in the peel, which can lead to different behaviours under bagging conditions (Yuan et al., 2012). Whatever the case, this changing behaviour suggested that the asymmetric rehydration of previously water stressed fruits is the main factor leading to pomegranate fruit-splitting (Galindo et al., 2014a).

The higher marketable yield in WSB than in WSNB was due to the high incidence of unmarketable fruits in WSNB plants caused by peel sunburn. This physiological disorder arises from the fruit peel being directly exposed to high sunlight, which burns the fruit surface, decreasing their appeal and rendering unmarketable fruits, with inevitable economic losses (Melgarejo et al., 2004; Yazici et al., 2005). Rabinowitch et al. (1974) indicated that peel sunburn seems to be mainly caused by the concurrence of two factors, heat and light, the threshold values of which are cultivar-dependent. However, it has not been established how much time is necessary to induce sunburn. Yazici and Kaynak (2009) showed that during July, August and September, when air temperature and solar radiation are higher than 30 °C and 600 Wm^{-2}, respectively, sunburn peel damage takes place. In this sense, it is important to underline that under our experimental conditions, daily maximum radiation values were above 600 Wm^{-2} every day and on 38 days the maximum daily air temperature was above 30 ºC, which could explain the high sunburn incidence in NB fruits.

From the statistical point of view, bagging induced a significant overall tendency of peel and arils to turn to a yellower colour, which supports the idea that this factor (bagging) affected fruit growth and ripening. However, it is important to consider that chromatic changes observed in peel and arils were of limited real significance because changes of less than two units do not cause perceptible visual differences (Navarro et al., 2011; Cano-Lamadrid et al., 2017; Galindo et al., 2015).

The decrease in TSS values due to bagging in WS plants would have been due to the partial protection of the bagged fruits, which would decrease fruit water loss and favoured water accumulation within the fruit. Moreover, a higher fruit water content would favour the translocation of soluble solids (Chonhenchob et al., 2011; Zhang et al., 2015). Similar results were obtained by Meena et al. (2016) in pomegranate fruits under coloured shade nets, by Amarante et al. (2002) in bagged pear fruits and by Seeley et al. (1980) in shaded apple fruits. However, Bentley and Viveros (1992) described how TSS increased in bagged apple fruits in only one of the two years of the experimental period, and Hofman et al. (1997) concluded that TSS values in mango fruits were not affected by bagging. In any case, the decrease in maturity index values by the bagging effect in FI fruits as a result of the maintenance of TSS and the increase in titratable acidity values, can be considered an unfavorable aspect because the overall consumer perception pomegranate fruit quality is related more to maturity index than to the soluble sugars content alone.

The decrease in antioxidant activity of pomegranate juice caused by water stress effect could be explained by the fact that water stress inhibits the biosynthesis of punicalagin (Mena et al., 2013), which significantly contributes to the antioxidant activity of pomegranate juice Tzulker (2007). The increase in antioxidant activity observed in FI fruits as a result of bagging effect contrasted with the results of Meena et al. (2016), who indicated that pomegranate fruits cultivated in open conditions have higher antioxidant activity than those grown under shade net, and hypothesized that probably plants use the induction of antioxidants as a protective measure to avoid peel sunburn. Also, Weerakkody et al.(2010) showed that sunscreen did not affect the total polyphenols content or the antioxidant activity of pomegranate juice. These different responses of antioxidant activity to bagging may be related to the characteristics of the bags in question, mainly light transmittance (Xu et al., 2010)1.

Only significant differences were detected in apple, pomegranate and fruity flavour notes as a result of water stress, but no significant differences were observed as result of the bagging. This experimental finding indicated that the chemical changes occurring in the pomegranate fruits were not reflected in significant changes in the organoleptic attributes. However, it is also possible that the heterogeneity of the fruits can mask the small differences in the sensory attributes due to the bagging factor; this masking effect of fruit heterogeneity on quality parameters is quite frequent in fruits and vegetables. This is, the increase in TSS and maturity index values as a result of water stress and the decrease in the same values as a result of bagging were not related with changes in the corresponding sensory attributes (sweetness, pomegranate, apple, pear, fruity dark, grape, berry, cranberry and floral notes). It is important to mention that the fact that no significant effect of bagging on the sensory quality is a positive result and indicated that the inner quality of the pomegranates was as good as that of the control samples.

Considering the chitosan spraying assay, the high Ψ_{stem} values in FI plants throughout the experimental period suggested that control plants were under non-limiting soil water conditions (Galindo et al., 2013, 2014b). In contrast, at the end of the water withholding period, the low Ψ_{stem} values indicated that WS plants were experiencing a relatively strong water stress situation (Galindo et al., 2013), even though these minimum Ψ_{stem} values were not reached progressively due to the rain that fell during the experimental period. The absence of a chitosan effect on Ψ_{stem} values in FI and WS at any time point examined (data not shown), agrees with the results of Yang et al. (2009), who concluded that spraying chitosan, at any of the concentrations tested, had no effect on apple predawn leaf water potential changes induced by drought.

As expected, WS fruits were smaller than those in FI plants because of pomegranate fruit growth and fruit ripening are critical phenological periods from the yield point of view since water deficit affects total yield and fruit size (Mellisho et al., 2012; Laribi et al., 2013; Galindo et al., 2017). Moreover, the fact that withholding irrigation water effect was larger on marketable yield than total yield was due to the high fruit cracking and/or splitting incidence in WS treatment fruits, which is directly linked to the fruit water status at the end of fruit growth and ripening phase. When previously water stressed pomegranate fruits are rehydrated, the increase in turgor pressure is higher in the arils than in the fruit peel, leading to fruit incidence of cracking and/or splitting physiopathies (Galindo et al., 2014a; Rodríguez et al., 2018).

It is well known that chitosan is an ideal fruit preservative coating due to its film-forming and physical and biochemical properties (Park et al., 2002; Romanazzi et al., 2002; Shiri et al., 2013). The plant response to exogenous chitosan application depends not only on its chemical characteristics and the concentration of the chitosan molecules (Lin et al., 2005; Limpanavech et al., 2008; Kananont et al., 2010) but also on the plant material (Ohta et al., 2004) and their developmental stage (Pornpienpakdee et al., 2010). Whatever the case, the semipermeable layer formed by polysaccharides like chitosan coating modifies the internal atmosphere of the fruit, and due to their hygroscopic properties enable the formation of a water barrier and consequently reduce external water transfer and decrease the rate of respiration, among other effects (Zhang and Quantick, 1997; Zhang et al., 2011). For these reasons, the reduction of fruit cracking and/or splitting incidence in WS fruits as a result of chitosan spraying may have been due to an effective antitranspirant effect of the chitosan coating, which led to a more conservative water use in treated fruits (WSQ) than in non-treated fruits (WSNQ). Hence, when irrigation was resumed, the increase in aril turgor pressure in WSQ fruits was lower than in WSNQ, so that the pressure of the arils on the peel (which favours cracking and/or splitting) was lower in WSQ fruits than in WSNQ fruits. On the other hand, the fact that sunburn incidence decreased in FIQ and WSQ fruits in relation to that observed in FINQ and WSNQ, respectively, could be attributed to the characteristics of the chitosan film around the fruit, which would act as a physical barrier against overall heat stress, reflecting harmful UV and IR radiation away from plants and, consequently, preserving fruit peel from sunburn.

The effect of water withholding and chitosan spraying on pomegranate peel and arils colour were low. In this sense, the first factor did not affect arils colour, but induced an increase in peel brightness, whereas the second factor induced less reddish and duller arils and a less yellowish and more reddish peel. Furthermore, the interaction between chitosan spraying and water withholding treatments led to the peel from

FIQ fruits being less yellow than in FINQ, while the peel in FINQ fruits was brighter than that of WSQ fruits. This interaction also induced FI arils to be lighter and less red through a chitosan effect. With regard to the effect of the first factor, in a comparison of different withholding irrigation water treatments during fruit growth and late ripening Galindo et al. (2017) described a significant effect of water stress on pomegranate peel colour because *L*, b* and H°* values of the peel tended to decrease with accumulated water stress effect. These different behaviours could be attributed to the fact that the response to water stress of fruit of a specific cultivar depends not only on the water stress level, but also of the phenological phase at which it takes place, its duration and its development rate (Galindo et al., 2018; Rodríguez et al., 2018). As regards the second factor, Munhuweyi et al. (2017) observed a significant effect of the pomegranate arils cultivar in response to chitosan treatment during postharvest cold storage, because Wonderful cv. arils colour did not change, whereas Herskawitz cv. arils showed a less red and yellower colour, especially at the beginning of the storage period.

The increase in total soluble solids as a result of irrigation water withholding is in line with the results presented by Laribi et al. (2013) and can be attributed to the active hydrolysis of starch to sugars, whereas the increase in titratable acidity could be due to an increase in the conversion of soluble sugars into organic acids (Munhuweyi et al., 2017). The increases in total soluble solids and titratable acidity in pomegranate fruit can be considered as a positive characteristic from a consumer acceptance point of view (Martínez-Romero et al., 2013).

The increase in AA-ABTS$^+$ values through a chitosan effect agrees with the observations of Candir et al. (2018), who showed an increase in antioxidant capacity for pomegranate fruits treated with chitosan during cold storage. Similarly, Zahran et al. (2015) described an increase in antioxidant activity for pomegranate arils treated with irradiated chitosan during cold storage . However, the fact that chitosan increased AA-ABTS$^+$ values and did not affect TPC levels is not in line with the results of

Borochov-Neori et al. (2009), who indicated that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this respect, to fully understand the antioxidant capacity and bioactivity of pomegranate fruits treated with chitosan, it is important to take into consideration that antioxidant activity of pomegranate arils is due to anthocyanin content, ascorbic acid and phenolic acids, either or in combination, are responsible for antioxidant activity of pomegranate arils (Sarkhosh et al., 2009). So, complementary analysis of fatty acids (Alcaraz-Mármol et al., 2015), organic acids such as gallagic acid, ellagic acid and gallic acids (Kulkarni et al. 2004; Calín-Sánchez et al., 2013), punicalin and punicalagin (Kulkarni et al. 2004) should be conducted.

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9. Conclusions and Conclusiones

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Conclusions

1. Leaf turgor maintenance in persimmon plants exposed to mild water stress was due to stomatal control and the retention of water due to the high relative apoplastic water content.

2. Sap flow measurements in addition to provide continuous and automated registers of the persimmon water status in real time, are the most suitable method for estimating its water status.

3. Quince plants cultivated under our experimental conditions were characterized by an extreme anisohydric behaviour.

4. The quince leaf conductance behaviour suggested that stomatal aperture was controlled directly by a hydroactive mechanism alone.

5. In response to water stress, quince plants developed active osmotic adjustment (stress tolerance mechanism), which probably contributed to leaf turgor maintenance during the experiment and, consequently, to a good leaf productivity.

6. The low quince leaf apoplastic water fraction under water stress could be considered as another drought tolerance characteristic because if accumulation of water in the apoplasm is avoided a steeper gradient in water potential between the leaf and the soil can take place under water stress, thus favouring water absorption.

7. The linear fruit growth phase was seen to be a critical quince phenological period because water stress decreased marketable yield and changed fruit chemical characteristics, mainly most of the volatile compounds identified, increased the total soluble solids

content and total polyphenolic content and decreased antioxidant activity.

8. The high levels of total polyphenolics, regardless of the quince fruit water status, suggested that quinces can be used as an important source of natural antioxidants.

9. The quince fruit sensory analysis indicated that water deficit during fruit growth increases peel colour intensity and improves fruit flesh texture because the main drawbacks for its consumption as fresh fruit (corkiness and fibreness) severely decreased, whereas crunchiness increased.

10. Pomegranate fruit ripening is a critical period from the yield point of view because irrigation is essential during most of this phenological period if maximum yields are to be achieved. Nevertheless, a very short irrigation restriction period (around 6 days) at the end of ripening saves irrigation water and enhances the fruits colouration and bioactive compound content without affecting marketable yield and fruit size and favouring an increase in fruit price.

11. Our results confirmed the hypothesis that there is no a linear correlation between pomegranate water stress and secondary metabolite contents, because mild water stress may lead to a reduction in plant growth and a higher concentration of secondary carbon metabolites, whereas under a more pronounced water stress carbon are preferentially allocated to the synthesis of primary metabolites to the detriment of secondary metabolites

12. Preharvest pomegranate fruit spraying with chitosan had little effect on peel and aril colour and the chemical characteristics of the arils. Some of these effects were negative such as the reduction in fruit

weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel physiopathies, which would considerably improve the returns of pomegranate growers.

13. Preharvest pomegranate fruit bagging may have certain negative effects in terms of slowing down fruit growth and ripening. However, such negative aspects may be outweighed by other very important commercial benefits such as the increase in antioxidant activity and the reduction in peel sunburn.

14. This would explain some contradictory results in the literature on fruit responses to preharvest bagging. Future research into pomegranate fruit bagging should take into consideration that in addition to the specific characteristics of the bags, other complementary factors and environmental conditions may interact and so affect the fruit response to bagging. These complementary factors would include the plant cultivar, the phenological period during which fruits are bagged, the material of the bags, the duration of fruit exposure to natural light from the time the bags are removed to fruit harvesting and cultivation practices (irrigation, pruning and thinning characteristics, among others).

Conclusiones

1. El mantenimiento de la turgencia celular en hojas de caqui bajo estrés hídrico suave parece basarse en el desarrollo de un progresivo control estomático y la alta retención de agua debido a los altos contenidos relativos de agua apoplástica.

2. Las medidas de flujo de savia constituyen un método altamente eficaz para conocer el estado hídrico del caqui en tiempo real, y, además, constituyen el método más fiable de diagnóstico de su estado hídrico.

3. Los membrillos exhibieron una conducta claramente típica de plantas anisohídricas extremas bajo las condiciones experimentales estudiadas.

4. El comportamiento de la conductancia foliar bajo las distintas condiciones de riego estudiadas sugirieron que la apertura estomática viene controlada de forma prácticamente exclusiva por un mecanismo hidroactivo.

5. Los membrilleros bajo déficit hídrico bajo condiciones de déficit hídrico desarrollaron un mecanismo de ajuste osmótico activo, mecanismo de tolerancia al estrés, el cual contribuyó al mantenimiento de la turgencia celular en todas las situaciones y, por tanto, una buena productividad.

6. Los bajos niveles de agua apoplástica en condiciones de déficit hídrico puede considerarse como otra característica de tolerancia a la sequía, ya que al evitar la acumulación de agua en el apoplasma bajo déficit hídrico se generaría un mayor gradiente de potencial hídrico entre la hoja y el suelo, favoreciendo la absorción de agua.

7. La fase de crecimiento rápido del membrillo resultó claramente crítica ya que bajo déficit hídrico disminuye la producción comercial y se modifican las características de los frutos, esencialmente la mayoría de los compuestos volátiles y se disminuye la actividad antioxidante, aunque se aumenta el contenido en sólidos solubles y polifenoles.

8. El hecho de que bajo todas las condiciones de riego los polifenoles totales sean altos, confirma el valor de estos frutos como fuente natural de antioxidantes.

9. El análisis sensorial de los membrillos bajo déficit hídrico mostró un color de la piel más intenso y mejor textura de la pulpa, ya que disminuye su fibrosidad y acorchado, aumentando su textura crujiente.

10. El periodo fenológico de maduración de la granada resultó ser claramente crítico desde el punto de vista de la producción. No obstante, la supresión del riego durante un corto periodo de tiempo, unos 6 días, antes de la cosecha posee consecuencias claramente favorables ya que ahorra agua, incrementa la coloración de los frutos y aumenta el contenido de compuestos bioactivos sin afectar a la producción comercial y el tamaño del fruto, por lo que puede favorecer un mayor precio de la cosecha.

11. Los resultados obtenidos confirmaron la hipótesis de que no existe una relación lineal entre el nivel de estrés hídrico y el contenido de metabolitos carbonados secundarios. Bajo situaciones de estrés moderado puede reducirse el crecimiento vegetativo y aumentarse el contenido de metabolitos secundarios carbonados, mientras que a niveles más severos de déficit hídrico el carbono se destina

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preferencialmente a la síntesis de metabolitos primarios en detrimento de los metabolitos secundarios.

12. La utilización de tratamientos previos a la recolección a base de quitosanas a la parte aérea de los granados tuvo mínimos efectos sobre la piel y los arilos de los frutos, pero algunos de ellos como la reducción del peso unitario y la reducción del coloreado de los frutos puede pueden considerarse como negativos. Sin embargo, el aumento de la actividad antioxidante y la importante reducción en la incidencia de fisiopatías pueden considerarse como aspectos altamente positivos en beneficio directo de los agricultores.

13. El embolsado de las granadas precosecha también presentó algunos aspectos negativos, tales como la ralentización del crecimiento y la maduración del fruto. Sin embargo, el aumento de la actividad antioxidante y la reducción del albardado pueden ser claves para optimizar la calidad de la granada y la rentabilidad del cultivo.

14. El embolsado de granadas precisa de investigaciones posteriores que aborden las características de las bolsas y su interacción con otros factores complementarios y condiciones ambientales. A este respecto cabría abordar el efecto del embolsado en distintos cultivares, momento de colocación de las bolsas, momento de retirada de las mismas, así como algunas prácticas culturales (riego, poda, y aclareo, entre otras).

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