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PRECISION IRRIGATION IN CITRUS WITH DIFFERENT WATER QUALITIES

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LIST OF ABBREVIATIONS AND SYMBOLS

CARM	Comunidad Autónoma de la Región de Murcia
CREM	Centro Regional de Estadística de Murcia
CTI	Canopy thermal index
CWSI	Crop water stress index
DOY	Day of year
ET_0	Reference crop evapotranspiration
ET_c	Crop evapotranspiration
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations. Statistics division
FDR	Frequency domain reflectometry
FG	Fruit growth
FI	Full irrigation
FS	Flowering sprouting
g_s	Stomatal conductance
IPCC	Intergovernmental Panel on Climate Change
MAPA	Ministerio de Agricultura Pesca y Alimentación
MDS	Maximum daily shrinkage
MedECC	Mediterranean Experts on Climate and environmental Change
MNTD	Minimum trunk diameter
MXTD	Maximum trunk diameter
PAR	Photosynthetically active radiation
P_n	Net photosynthesis
PRD	Partial root-zone drying
r^2	Coefficient of determination
RDI	Regulated deficit irrigation
RH	Relative humidity
RW	Reclaimed water
SIAM	Sistema de Información Agrario de Murcia

SMP	Soil matric potential
Sp.	Species
SPAC	Soil-plant-atmosphere continuum
SWC	Soil water content
T_a	Air temperature
T_c	Canopy temperature
T_c-T_a	Canopy to air temperature difference
$(T_c$-$T_a)$LL	Canopy to air temperature difference lower limit
$(T_c$-$T_a)$UL	Canopy to air temperature difference upper limit
TDR	Time domain reflectometry
TDV	Trunk diameter variations
TSS	Total soluble solids
TW	Transfer water
UN	United Nations
VPD	Vapor pressure deficit

θ_v	Volumetric soil water content
Ψ_{leaf}	Leaf water potential
$\Psi_{predawn}$	Predawn water potential
Ψ_{stem}	Stem water potential

CHAPTER I: GENERAL ABSTRACT

1. Resumen

El cambio climático hará que la temperatura de la Tierra aumente 2,2 ◦C de aquí a 2040. Se prevé que este aumento de la temperatura del aire vaya acompañado de una reducción de las precipitaciones, lo que aumentará la intensidad y la frecuencia de los episodios de sequía. Paralelamente, la población mundial es más de tres veces mayor que a mediados del siglo XX y seguirá aumentando en los próximos años, lo que incrementará la demanda de agua para la producción de alimentos. En la actualidad, la producción mundial de alimentos mediante regadío representa más del 40 % del total y sólo utiliza alrededor del 20 % de la superficie destinada a este fin. Los cítricos son una de las principales especies cultivadas en todo el mundo, repartidas en más de 140 países. Esta especie depende del riego y tiene una demanda de agua media-alta. Para hacer frente a la sequía, la agricultura de regadío se ve obligada a realizar los máximos esfuerzos para mejorar la eficiencia en el uso del agua.

En este contexto, la presente tesis doctoral tiene como objetivo evaluar la sostenibilidad del riego de precisión de cultivos de cítricos (pomelo y lima), con especial atención al estudio de la evapotranspiración del cultivo (ET_c) y al uso de indicadores del estado hídrico de suelo y planta. Para alcanzar estos objetivos se han llevado a cabo varios experimentos (**Artículos del I al IV**).

Un buen conocimiento de la ET_c es necesario para decidir "cuándo" regar y "cuánta" agua aplicar. En el **Artículo I**, se calculó la ET_c de limeros jóvenes por el método del balance hídrico del suelo utilizando lisímetros de pesada. A lo largo del ciclo de cultivo, la ET_c fue más alta en Julio (2,56 L planta^{-1}día^{-1}), debido al elevado consumo de agua durante la última fase de crecimiento del fruto. Cuando los cambios en la biomasa de la lima fueron insignificantes, las variaciones en el peso de las macetas servían para validar la ET_c calculada por el método del balance hídrico (r^2=0,92***). En este artículo, se propone un prototipo de lisímetro de pesada escalable, sencillo y robusto, para árboles jóvenes cultivados en maceta, que proporciona una forma práctica y rentable de medir la ET_c durante todo el año.

En limeros jóvenes cultivados al aire libre y bajo malla de sombreo se midieron indicadores discretos del estado hídrico de la planta, como el potencial hídrico del tallo (Ψ_{stem}), la conductancia estomática (g_s) y la fotosíntesis neta (P_n), así como indicadores continuos como la temperatura de la copa (T_c) (**Artículos II y III**). En el **Artículo II**, el

sombreado incrementó un 54 y 37 % la g_s y la P_n diaria acumulada, respectivamente. Además, bajo malla se incrementó el Ψ_{stem} en 0,05 MPa y se redujeron los valores de la diferencia de temperatura entre la copa y el aire (T_c-T_a) en 1,7 ◦C según los **Artículos II y III**. Estos hechos apuntaron un mejor comportamiento fisiológico de los limeros en condiciones de sombreado. En el **Artículo III**, se evaluó el efecto combinado del sistema de cultivo y el riego deficitario en los indicadores de estado hídrico del suelo y de la planta. Los resultados indicaron que cuando el estrés hídrico del suelo fue moderado, las condiciones de sombreado amortiguaron el efecto negativo sobre el estado hídrico del suelo y de la planta, permitiendo posponer el déficit hídrico. Sin embargo, cuando el estrés hídrico del suelo fue severo, se observaron valores igualmente bajos de estos indicadores tanto en campo abierto como en condiciones de sombreado. Además, se proponen dos nuevos índices basados en la T_c, que mostraron una respuesta diferente según el sistema de cultivo y el tratamiento de riego considerado.

La programación del riego basada en indicadores del estado hídrico del suelo y de la planta permite ajustar la dosis de riego a las necesidades hídricas del cultivo. En esta tesis, el riego de los limeros se gestionó automáticamente, en base a valores umbrales del contenido de agua del suelo medidos en la zona radicular activa. El uso de este protocolo de riego promovió un estado hídrico óptimo de la planta, como se observó en los limeros cultivados en maceta (**Artículo I**) y en el suelo (**Artículos II y III**). En cuanto a los indicadores del estado hídrico de la planta, la medida de la T_c a distancia y en tiempo real supone una gran ventaja para la gestión del riego. En este sentido, en el **Artículo III**, se propusieron valores umbrales de la T_c-T_a de 2,4 y 0,8 ◦C para limeros bien regados cultivados al aire libre y bajo malla de sombreo, respectivamente. En el **Artículo IV**, se evaluó la robustez de varios índices basados en la T_c para estimar la g_s en pomelos adultos regados con diferentes cantidades (riego completo y deficitario) y calidades de agua (trasvase y agua regenerada) de riego durante dos estados fenológicos (floración-brotación y crecimiento del fruto). Además, se comprobó la capacidad de diferentes índices térmicos para determinar la g_s en diferentes intervalos de datos térmicos y en diferentes condiciones de déficit de presión de vapor (VPD) y de radiación fotosintéticamente activa (PAR). Los resultados sugirieron que los índices térmicos y la g_s tenían el mayor grado de correlación cuando los datos térmicos se promediaron a 60 min, a VPD entre 0 y 3,5 kPa, y a PAR 1200 μmol m^{-2} s^{-1}. En estas condiciones, los árboles con riego deficitario y los regados con agua regenerada fueron los que mostraron

las mejores correlaciones entre los índices térmicos y la g_s. El índice de estrés hídrico del cultivo (CWSI) y la T_c-T_a estimaron mejor la g_s durante la floración-brotación, y los valores absolutos de T_c durante el estado fenológico de crecimiento del fruto, independientemente de la calidad y cantidad de agua aplicada. Esto indicó que durante floración-brotación la T_c dependía principalmente de las condiciones climáticas más que del estado hídrico de la planta, mientras que durante el período de crecimiento del fruto la T_c estaba más influenciada por factores intrínsecos de la planta (apertura estomática). En general, los índices térmicos han demostrado ser buenos indicadores del estado hídrico de la planta para una gestión precisa del riego en cítricos.

2. Abstract

In the coming years, climate change will cause the earth's temperature to rise 2.2 ◦C by 2040. This increase in air temperature is expected to be accompanied by a reduction in precipitation, which will increase the intensity and frequency of drought events. In parallel, the world's population is more than three times larger than it was in the mid-twentieth century. It will continue increasing in the subsequent years, driving up water demand for food production. Currently, global food production from irrigation represents more than 40 % of the total and uses only about 20 % of the land area designated to this end. *Citrus* is one of the main species cultivated worldwide, spread in more than 140 countries. This species is irrigation-dependent, and it has a medium-high water demand. In order to cope with this water scarcity, irrigated agriculture is forced to make utmost efforts to improve water use efficiency.

In this context, this thesis is aimed to assess the sustainability of precise irrigation of citrus crops (grapefruit and lime), with special attention to the study of crop evapotranspiration (ET_c) and the use of soil and plant water status indicators. To achieve these objectives several experiments were carried out (**Articles I to IV**).

A sound knowledge of ET_c is necessary to decide 'when' to irrigate and 'how much' water to apply. In **Article I**, young lime's ET_c was calculated by soil water balance method using drainage-weighing pot-lysimeters. Along the crop cycle, ET_c was the highest in July (2.56 L $plant^{-1}day^{-1}$), due to the elevated water consumption during the last fruit growth stage. When lime tree biomass changes were negligible, pot weight variations served for ET_c-water balance validation ($r^2=0.92^{***}$). A prototype of a simple and robust scalable drainage-weighing lysimeter for young potted trees was proposed, which provided a practical and cost-effective way to measure ET_c throughout the year.

Discrete plant water status indicators, as stem water potential (Ψ_{stem}), stomatal conductance (g_s) and net photosynthesis (P_n), as well as continuous ones like canopy temperature (T_c) were measured on lime trees grown in shaded and in open-field conditions (**Articles II and III)**. In **Article II**, shading increased by 54 and 37 % daily accumulated leaf g_s and P_n, respectively. Moreover, in shaded conditions Ψ_{stem} was increased by 0.05 MPa and canopy to air temperature difference (T_c-T_a) values were reduced by 1.7 ◦C according to **Articles II and III**. These facts pointed to a better physiological performance of lime trees under shading conditions. In **Article III**, the

combined effect of cropping system and deficit irrigation on soil and plant water status indicators was evaluated. The results indicated that when soil water stress was moderate, shading conditions cushioned the negative effect on soil and plant water status, allowing to postpone water deficits. However, when soil water stress was severe, similar low values of these indicators were observed in open-field and in shaded conditions. Additionally, two new thermal-based indices were proposed, which showed a different response by cropping system-irrigation treatment considered.

Irrigation scheduling based on soil and plant water status indicators allows adjusting the irrigation dose to crop water needs. In this thesis, irrigation of lime trees was automatically managed, based on soil water content threshold values measured in the active root zone. This irrigation protocol promoted an optimal plant water status, as proved in pot grown (**Article I**), and in field grown (**Articles II y III**) lime trees. Regarding plant water status indicators, remote and real-time T_c measurement supposes a great advantage for irrigation management purposes. In this sense, in **Article III**, T_c-T_a threshold values of 2.4 and 0.8 ◦C were proposed for full irrigated lime trees in open-field and shaded conditions, respectively. In **Article IV**, the robustness of several thermal-based indices to estimate g_s for different irrigation strategies (full and deficit irrigation), water sources (transfer and reclaimed water) and phenological stages (flowering-sprouting and fruit growth) in grapefruit trees were evaluated. In addition, the ability of thermal indices to determine g_s at different averaged thermal data intervals, vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) conditions, was tested. The results suggested that thermal indices and g_s had the highest degree of correlation at 60 min thermal average data, VPD between 0 and 3.5 kPa and PAR 1200 μmol m^{-2} s^{-1}. In these conditions, trees under deficit irrigation and those irrigated with reclaimed water showed the best thermal indices-g_s correlations. Crop water stress index (CWSI) and T_c-T_a estimated g_s better during flowering-sprouting (FS), and T_c during fruit growth (FG), independently of quality of the irrigation source and the amount of water applied. This fact indicated that in FS stage T_c depended mainly on climatic conditions rather than on plant water status, whereas in FG stage it was more influenced by intrinsic plant factors (stomatal opening). Overall, thermal-based indices have demonstrated to be suitable plant water status indicators for precise irrigation management of citrus trees.

CHAPTER II: OBJECTIVES

Ongoing climate change will disrupt the hydrological cycle, which concurrently with other factors will reduce the availability of freshwater. Irrigation agriculture is the main water consumer, accounting for 70 % of the world's freshwater. Thus, in this water scarcity context, it is important to apply precision irrigation in order to supply crop water demand, reducing its negative effects.

In order to apply irrigation in a precise way, an accurate knowledge of crop evapotranspiration is needed. Similarly, the use of soil and plant water status indicators in the irrigation scheduling also contributes to this purpose. Among these indicators, the ones based on the soil water content and canopy temperature continuous measurements allow adjusting water applied in real-time supposing this a great advantage from an irrigation management point of view.

In the Mediterranean area, cropping techniques, such as shading or irrigation strategies, like regulated deficit irrigation, have been proposed to increase water use efficiency. In line with this, the use of alternative water sources, such as reclaimed water, also contributes to mitigate freshwater scarcity. The application of these techniques and strategies could affect plant and soil water status indicators behaviour, so this issue needs to be studied before using these indicators for irrigation management.

Within this framework, this thesis aims to integrate novel multidisciplinary approaches in the sustainability of precision irrigation of grapefruit and lime trees (two *Citrus* sp. with an added value), grown in Mediterranean climate, focussing on the study of crop evapotranspiration and the use of soil and plant water status indicators. For this purpose, the following specific objectives were proposed:

- Determine the water requirements of young lime trees by real-time monitoring of water balance parameters in pot-lysimeters (**Article I**).
- Establish soil (**Articles I, II, and III**) and plant (**Article III**) water status indicators threshold values for precise irrigation scheduling of lime trees.
- Compare discrete plant water status indicators with continuous thermal-based indices in lime and in grapefruit trees (**Articles II, III and IV**).
- Assess the effect of the cropping system (shaded *vs.* open-field) and soil water deficit on soil and lime tree water status (**Articles II and III**).

- Evaluate the effect of regulated deficit irrigation and reclaimed water on the robustness of thermal-based indices to estimate stomatal conductance in grapefruit trees (**Article IV**).

CHAPTER III: STATE OF THE ART

1. *Citrus* genus

1.1. Taxonomy and origin

Citrus genus is one of the most widely cultivated plants around the world (Wu et al., 2018). This genus is taxonomy classified into *Geraniales* Order (Inglese and Sortino, 2019), *Rutaceae* Family (Salvo et al., 2010) and *Aurantioideae* Subfamily (Morton et al., 2003). Most *Citrus* species belong to the cross of four ancestral species: *Citrus reticulata* (mandarins), *Citrus maxima* (pummelos), *Citrus medica* (cidros) and *Citrus micrantha* (Luro et al., 2017) (Figure 1). From an agronomic point of view, the most important species are:

- Clementine (*C. clementina* Hort.)
- Grapefruit (*C. paradisi* Macf.)
- Key lime or Mexican lime (*C. aurantifolia* (Christm.) Swing)
- Lemon (*C. limon* (L.) Burn.)
- Mandarin (*C. reticulata* Blanco)
- Persian lime or Tahiti lime (*C. latifolia* Tan.)
- Pummelo (*C. maxima* (L.) Osb.)
- Satsuma (*C. unshiu* (Mak.) Marc.)
- Sour orange (*C. aurantium* L.)
- Sweet orange (*C. sinensis* (L.) Osb.)

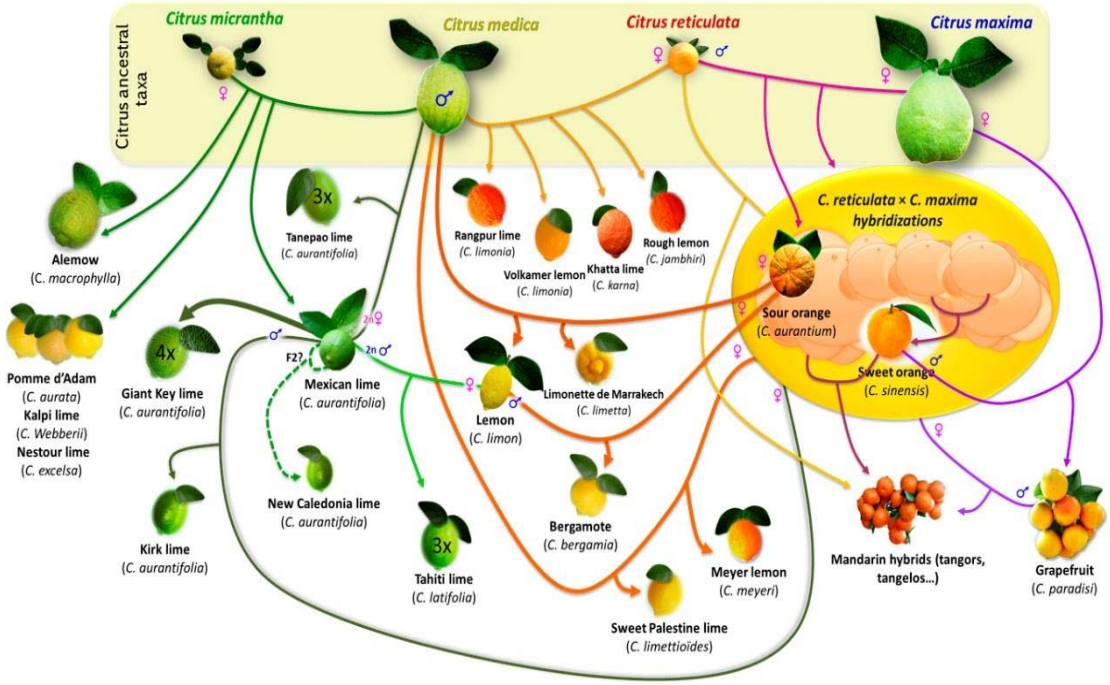

Figure 1. Genetic origin of the main *Citrus* species. Source: Luro et al. (2017).

Over the years, several authors have reported information on the geographical origin of *Citrus* species. Vavilov (1927) identified two origin centres: the Indo-Burna centre (Assam and Myanmar) and the Indo-Malayan centre (Indochinese Peninsula and the Malay Archipelago). Swingle and Reece (1967) suggested that most important cultivars of true *Citrus* appeared in an extensive region of Southeast Asia comprising: Northeast India, Myanmar and Southeast China. Recently, Talon et al. (2020) reported that the origin of *Citrus* genus is in a broad area comprising India, Southeast Asia, Australasia, Melanesia, and Polynesia.

1.2. Botanical traits

Citrus is an evergreen tree 3 to 6 m high. The underground part of the tree corresponds to the root system, composed by: (i) the main taproot, which anchors the plant to soil and (ii) lateral or secondary roots, responsible for the absorption of water and nutrients (Ancillo and Medina, 2015). The aerial part of the tree is formed by trunk and branches. Trunk is green and tender in young trees and brown and more rigid in adult ones. Branches are brown, irregularly shaped and often angled. These can be classified into primary and secondary ones, being leaves, flowers and fruits in the secondary ones. The leaf is compound by blade and petiole, which joins the blade with stem. Leaf blade is dark green

in the top and light green in the down. The shape of it can be elliptical, oval or lanceolate depending on *Citrus* species (Ancillo and Medina, 2015). The petiole is dark green and in some species as grapefruit or sour orange is winged. In most cases, leaves contain a solitary thorn at the petiole-stem joint. Flowers, also called 'azahar' (Mazorra-Manzano et al., 2013), are white or purple with a green calyx and a corolla of five petals containing 20 to 40 stamens. Fruit is a hesperidium (Tadeo et al., 2008) composed by: the exocarp or flavedo (peel), mesocarp or albedo and endocarp. The exocarp is rigid and contains essential oils and the mesocarp is white and spongy (Tütem et al., 2020). The endocarp comprises juice vesicles (Inglese and Sortino, 2019), which contain sugars and organic acids among other nutritional valuable substances. Some fruit characteristics, such as number of seeds and peel colour, depend on *Citrus* species.

1.3. Phenological stages

Plants' phenological growth stages can be classified using BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie, Germany) scale. This classification system was proposed by Bleiholder et al. (1989), and since then it has been widely used to describe the phenological stages of several herbaceous and woody crops (Lancashire et al., 1991; Pérez-Pastor et al., 2004; Sanz-Cortés et al., 2002). Agustí et al. (1995) proposed the BBCH scale for *Citrus* species, composed for the following principal growth stages:

0: Sprouting/Bud development
1: Leaf development
3: Shoot development
5: Inflorescence emergence
6: Flowering (Figure 2)
7: Development of fruit (Figure 2)
8: Maturity of fruit
9: Senescence, beginning of dormancy

Figure 2. Main flower and fruit phenological stages of Bearss lime tree (*C. latifolia* Tan.).

The main phenological stages can be grouped in vegetative and reproductive development:

1.3.1. Vegetative development

In Mediterranean areas, citrus vegetative growth is a discontinuous process, which includes flush periods alternating with phases of quiescence (Carvalho et al., 2020). This process englobes the growth of shoots, leaves, roots and trunk. Shoots growth takes place through sprouting, which, in *Citrus* species, occurs two to four times, often three during crop cycle. The first sprouting is the most important and starts at the end of winter and lasts throughout spring (Arenas-Arenas et al., 2021); the second one happens early in the summer and the last one in autumn. Leaves growth coincides with shoot elongation period and lasts two months. Root growth initiates with the development of a primary root from the radicular meristem of the embryo. As the main root lengthens, lateral roots appear from the primary one. Trunk growth is vertical until it reaches the height of about 1 m. Then, it emits lateral branches and on these the secondary ones and so on.

1.3.2. Reproductive development

Citrus is a profusely bloomy species that can produce more than 250,000 flowers per tree (Agustí et al., 2022). In Mediterranean areas, the main flowering occurs in spring, and some secondary flowerings may happen after autumn and summer flushes but with a reduced number of flowers. Flowering comprises the following stages: flower-bud induction, bud differentiation and anthesis (Valiente and Albrigo, 2004). The main flowering bud induction occurs in the second half of autumn and is controlled by a

stimulation received by the leaf that allows the transition from vegetative to reproductive status. Later, the differentiation of these buds occurs by mid-winter (February) when the buds release from dormancy and primordia begin to grow. Subsequently flower development takes place, and when the flowers are fully formed, its pollination leads to fruit. Fruit growth follows a sigmoidal curve with three well differentiated stages (Allen, 1979; Kim et al., 2021). The first stage (stage I) is approximately two months from anthesis to physiological fruit fall. It includes the phase of cell division, which leads to the increase in volume of the fruit endocarp, and the formation of juice vesicles. The stage II lasts several months (2 to 6 depending on *Citrus* species) and comprises the elongation cellular process (Iglesias et al., 2007). The increase in fruit size in this stage is mainly due to the development of locules or segments, which allow juice vesicles reaching their final size. In the last stage of fruit development (stage III), changes associated to fruit maturation occur, fruit growth slows down and peel colour change, because of chlorophyll degradation and an increase in the carotenoid synthesis. Also, fruit sugar content reaches stable levels in this stage (Batista-Silva et al., 2018).

1.4. Commercial varieties and rootstocks

Commercial citrus trees are composed by: i) variety, which comprised the upper part of the trunk to the canopy, and ii) rootstock, referring to the lower part of the trunk and roots. The main *Citrus* species cultivated are sweet orange (*C. sinensis*), mandarin (*C. reticulata*), lemon (*C. limon*), key lime or Mexican lime (*C. aurantifolia*), Persian lime or Tahiti lime (*C. latifolia*) and grapefruit (*C. paradisi*). Lime and grapefruit trees, the two *Citrus* species studied in this thesis, are briefly described:

Lime is a natural triploid result from a diploid gamete of *C. aurantifolia* 'Mexican' lime recombined with a *C. micrantha* × *C. medica* hybrid (Rouiss et al., 2018). There are acid and sweet lime cultivars. The most spread acid cultivars are the Mexican (*C. aurantifolia*) and Persian (*C. latifolia*). In this thesis, the variety of lime studied is Persian or also called 'Bearss', in honour of J. T. Bearss, who developed it in 1895 in California, USA. Bearss trees are vigorous and spiny (Figure 3) in comparison with the Mexican ones (Singh et al., 2021). The fruits are large with a green intense colour and a greenish yellow pulp. The total soluble solids (TSS) of Bearss lime juice ranges between 7 to 9 ◦Brix (Jittanit et al., 2013) and its acidity is around 2 % (Bisen et al., 2012).

Figure 3. Five-year-old Bearss lime tree (*Citrus latifolia* Tan.).

Grapefruit is one of the most recently known commercial *Citrus* species (De Moraes et al., 2007). It was firstly described in 1750 by G. Hughes who called it the forbidden fruit of Barbados. This hybrid seems to come from an accidental crossing event between pummelo (*C. maxima*) and sweet orange (*C. sinensis*) on these islands. For this reason, in 1828 J. Macfayden gave it the botanical name of *Citrus* x *paradisi* indicating its hybrid origin (Scora et al., 1982). Grapefruit commercial varieties are divided in two groups: white fleshed (Marsh) and pigmented (Ruby Red, Ruben Pink, Henderson, Ray Ruby, Star Ruby, Rio Red among others) (Barry et al., 2020). In this thesis, the variety of grapefruit studied is Star Ruby, which is one of the most commercial red-fleshed varieties cultivated (Gentile et al., 2020). Star Ruby trees are dense and medium size in comparison with other grapefruit varieties (Hueso-Martín and Cuevas-González, 2014) (Figure 4). The fruit usually presents skin areas with reddish colouration and an intense red pulp. The TSS of Star Ruby grapefruit juice ranges between 8 to 10 ◦Brix, and its acidity is around 1.8 to 2.4 % (Romero-Trigueros et al., 2017b).

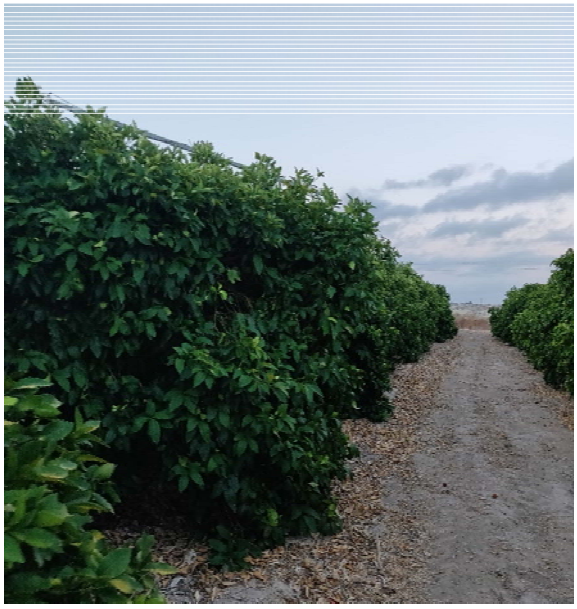

Figure 4. Fourteen-year-old Star Ruby grapefruit trees (*Citrus paradise* Macf.).

In citriculture, the selection of a good rootstock is a key factor, as it affects fruit weight and quality (Aguilar-Hernández et al., 2020). Also, rootstock influences plant resistance to biotic stresses as diseases and pests and in the tolerance to abiotic stresses such as flooding, drought and cold (Oustric et al., 2017). In Spain, prior to the arrival of the *citrus's* tristeza virus (CTV), sour orange was the main rootstock used (Legua et al., 2018). Yet as a consequence of its high sensibility to this disease, the use of alternative rootstocks is promoted. These are some of rootstocks used in citriculture:

- ***Citrus macrophylla*** is a hybrid of *C. micrantha* and *C. medica* (Curk et al., 2016). This rootstock induces vigorous trees with a high crop load, and good-quality and large fruits (Bowman and Joubert, 2020). It is tolerant to salinity as it excludes the transport of saline ions (as Cl^- and Na^+) from root to plant (Ruiz et al., 2018). This rootstock is also tolerant to boron rich soils. Nevertheless, it is susceptible to severe CTV strains, when it is used in other varieties different to lemon. In turn, *C. macrophylla* is sensitive to water deficit conditions in comparison with other rootstocks. Despite this, this rootstock is commonly used in California, and it has become popular in Spain and Morocco, where water with high salinity levels is used for irrigation purposes.
- **Carrizo** is used in the main citrus-producing countries: Spain, South Africa, Australia, California and Italy (Morales-Alfaro et al., 2021). It provides a medium tree vigour and fruit size with an excellent internal fruit quality, but a lower crop

load than *C. macrophylla*. This rootstock is sensitive to salinity, soil B enrichment (Aparicio-Durán et al., 2021) and chlorosis (Martínez-Cuenca et al., 2016). However, it is more tolerant to deficit irrigation conditions than *C. macrophylla*.

- **Forner-Alcaide 5 (F-A 5)** induces lower tree size but higher yield than Carrizo. It is resistant to CTV and nematodes and tolerant to calcareous, salinity and flooded soils (Priyadarshan and Mohan-Jain, 2009).

Other rootstocks used in citriculture are Cleopatra Mandarin, Citrange Troyer, *Citrus volkameriana* and Citrumelo CPB (González, 2017). In this thesis, the rootstock used is *C. macrophylla,* due to its great resistance of soil salinity and its high crop load and good-quality and large fruits.

1.5. Citrus production and exportations

Citrus species are widely cultivated in more than 140 countries (Gentile et al., 2020). Europe is one of the main citrus producers with an annual production of 11.8 Mt (FAO, 2021). Spain is the European leader (Figure 5), accounting for 58.7 % of the total citrus production (FAOSTAT, 2020). In fact, in this country, the surface designated to this crop is 296477 ha, being one of the most cultivated agricultural species (MAPA, 2021).

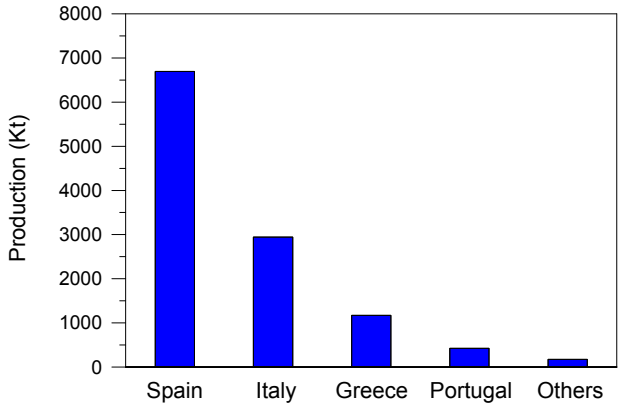

Figure 5. Main European citrus producers in 2020. Source: Food and Agriculture Organization of the United Nations. Statistics division (FAOSTAT).

Spanish production of this crop is concentrated mainly in Comunidad Valenciana, Region of Murcia and Andalucia (MAPA, 2021). In the Region of Murcia, citrus is a key crop, comprising 54.1 % of the total production of woody trees (CREM, 2021) (Figure 6).

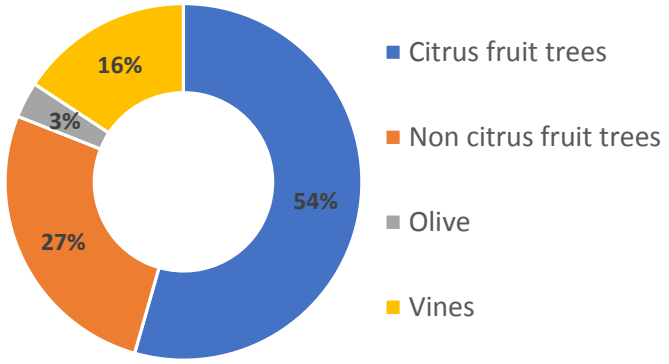

Figure 6. Distribution % of woody crops production in the Region of Murcia in 2021. Source: Centro Regional de Estadística de Murcia (CREM).

In the global context, lime and grapefruit are not the main *Citrus* species cultivated (FAO, 2021). Despite this, Spanish grapefruit production is relevant, accounting for 76.6 % of the total European production of this crop (FAOSTAT, 2020). Additionally, production of lime in Spain, although scarce, has been gaining importance in the last few years (CIRAD, 2021). Currently, much of Spanish grapefruit and lime production comes from the Region of Murcia (CARM, 2020). In fact, grapefruit and lime production in the Region of Murcia in 2021 was 34999 and 820 t, respectively, being the highest in recent years (CREM, 2021) (Figure 7).

In Spain, citrus exportations (4043.9 kt) are more significant than its importations (146.5 kt). Indeed, this country is Europe's leading exporter of this crop (FAOSTAT, 2020). The Spanish citrus exportations are destined principally to Germany and France, with annual figures of 1105.6 and 787.6 kt, respectively (MAPA, 2021).

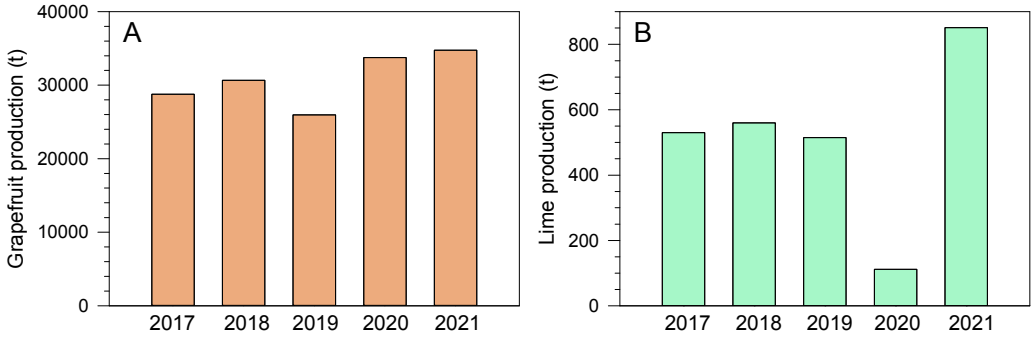

Figure 7. Grapefruit (A) and lime (B) production in the Region of Murcia during the period from 2017 to 2021. Source: Centro Regional de Estadística de Murcia (CREM).

The main *Citrus* species exported in Spain is orange, comprising 44.1 % of the total (MAPA, 2021). The international trade of grapefruit in this country is considerably lower than the orange one, but it accounts 91.9 % of the European trade of this crop. In turn, Spanish lime exportations, even though marginal, are expected to increase in the coming years (CIRAD, 2021). The Region of Murcia is the supplier of much Spanish grapefruit and lime exported (CREM, 2021).

2. Water management in a scarce context

The on-going climate change context favours global warming especially in Mediterranean areas. Nowadays, in these areas, average annual air temperature (T_a) is approximately 1.5 ◦C higher than during the preindustrial period (1880-1899) and is expected to have increased 2.2 ◦C by 2040 (MedECC, 2019) (Figure 8).

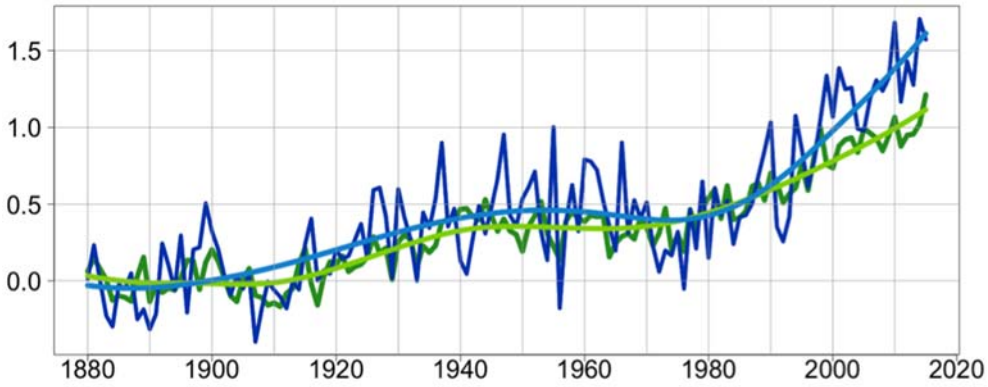

Figure 8. Warming of the atmosphere (annual mean temperature anomalies with respect to the period 1880-1899), in the Mediterranean Basin (blue lines, with and without smoothing) and for the globe (green lines, with and without smoothing). Source: Mediterranean Experts on Climate and Environmental Change (MedECC).

This increase in T_a is expected to be accompanied by a reduction of 10-20 % in precipitations (IPCC, 2021), increasing the intensity and frequency of drought events. In the Region of Murcia, this water scarcity scenario will be aggravated, since mean annual T_a is already up to 18 ◦C and precipitation is already low, being less than 400 mm per year (SIAM, 2021).

In parallel, the world population is more than three times larger than it was in the mid-twentieth century (UN, 2022), driving up the demand of water for food production. Agricultural production is highly dependent on water availability to ensure an optimal crop yield and fruit quality (Capra et al., 2011; Iniesta et al., 2009). Therefore, in this

water scarcity context, it is key to increase the precision of irrigation in agriculture, to avoid negative effects on the crops. In this sense, scheduling irrigation based on water status indicators contributes to this end.

2.1. Water status indicators

Water flows from soil to atmosphere throughout the plant, which has been termed soil-plant-atmosphere continuum (SPAC) (Philip, 1966). Knowledge of where and how much water is available at each point of the SPAC can be explained by soil and plant water status indicators. The most commonly used are described below.

2.1.1. Soil-based water status indicators

The soil water status can be assessed by the gravimetric method. This method consists in taking a soil sample and weighing it, oven drying, and after that reweighs again (Lunt et al., 2005). The water content in the soil sample is expressed as the weight loss. Traditionally, the gravimetric method has been widely used in the determination of soil moisture. Nevertheless, the use of this method has numerous disadvantages like, it does not permit measuring soil moisture on a continuous basis and it is destructive and time-consuming.

Soil moisture can be also measured with neutron probes, which has numerous advantages as the high speed and accuracy of its measurements (Yu et al., 2021). However, radiation hazards to health and certain safety concerns limit the use of these probes in agriculture. The use of soil water content or soil matric potential sensors allows determining real-time monitoring the soil water status (Vera et al., 2021).

2.1.1.1. *Soil water content*

Soil water content (SWC) sensors measure soil moisture indirectly based on the soil dielectric constant or in the H^+ content. Nowadays, SWC sensors most used in agriculture are based on the real soil dielectric constant, estimated by frequency domain reflectometry (FDR) and time domain reflectometry (TDR) (Table 1).

FDR sensor operation is based on the study conducted by Hilhorst et al. (1996). These sensors measure the SWC indirectly by determining the bulk dielectric constant from frequency variations of an electromagnetic pulse propagated into the soil (Hardie, 2020). One drawback in the use of these sensors is that their calibration process is more extensive than the TDR ones, due to the complexity of generating an electrical field for the different soil types. Despite this, they are currently used as a decision-making tool for

irrigation scheduling in agriculture (Conesa et al., 2021; Gasque et al., 2016; Martí et al., 2013). Instances of these sensors are Theros, EnviroSCAN, EnviroPro among others (Table 1).

The theoretical background of TDR sensors is based on the research conducted by Fellner-feldegg (1968). These sensors determine soil dielectric permittivity by measuring the delay in time between the incident and reflected electromagnetic pulses (Suchorab et al., 2014). The travel time of the wave through the sensor is a function of the apparent or effective permittivity of bulk soil. This travel time is translated to SWC by using the Topp's equation (Topp et al., 1980). Currently, TDR sensors are also widely accepted for agricultural irrigation management (Datta et al., 2018; Marković et al., 2021). TDR 315H and SoilVUE are examples of TDR sensors (Table 1).

Table 1. Main current soil water content sensors. Source: Vera et al. (2021).

Technique	Brand	Model	Design	Interface	Website
(i) TDR	Acclima, Inc.	TDR 315H TDR 310H TDR 305H	Parallel rods & Portable	SDI-12	www.acclima.com
	Campbell Scientific, Inc.	SoilVUE	Screw probe with cylindrical metal rings	SDI-12	www.campbellsci.com
		CS650, CS655	Parallel rods & Portable	SDI-12, RS232	
(ii) TDT	Acclima, Inc.	Acc TDT	Parallel rods & Portable	SDI-12	www.acclima.com
	Vegetronix, Inc.	VH400	Prong & Portable	0 - 3 V	www.vegetronix.com
	OnFarm Data Ltd.	Aquaflex	Belt (3 m)	SDI-12, 4-20mA	www.aquaflex.co.nz
	RioT Technology Corp.	GroPoint	Probe, Parallel rods & Portable	SDI-12, 4-20mA, RS-485	www.gropoint.com
(iii) Capacitance	Sentek Sensor Technologies	EnviroSCAN	Probe with cylindrical metal rings	RS232, RS485, SDI-12, 0-5 V	www.sentektechnologies.com
		Drill & Drop	Encapsulated probe		
	Meter Group Devices, Inc.	5TE	Parallel rods & Portable	RS232, SDI-12	www.metergroup.com
		10 HS	Parallel rods	300 - 1250 mV	
		Teros 12	Parallel rods & Portable	SDI-12	
	AquaCheck, Pty Ltd.	AquaCheck	Encapsulated probe cylindrical metal rings	SDI-12, RS-485	www.aquacheck.co.za
	Entelechy Pty Ltd.	EnviroPro	Encapsulated probe cylindrical metal rings	SDI-12	www.enviroprosoilprobes.com
(iv) Impedance	Stevens Water Monitoring Systems, Inc.	Hydraprobe	Parallel rods & Portable	SDI-12	www.stevenswater.com
	Delta-T Devices Ltd.	PR2 Profile	Encapsulated probe cylindrical metal rings	SDI-12, 0 - 1 V	www.delta-t.co.uk
		ML3 ThetaProbe	Parallel rods & Portable	0 - 1 V	

2.1.1.2. Soil matric potential

Soil matric potential (SMP) sensors measure soil moisture as a function of the force with which water is held by the soil matrix (Kukal et al., 2014). The SMP can be measured using tensiometers, gypsum blocks or granular matrix among other sensors (Hardie, 2020). The tensiometer consists of a porous cup, generally of ceramic material, connected through a tube to a manometer. Its low price makes this sensor one of the most widely used in the agricultural sector for the measure of SMP (Montesano et al., 2015). Yet, the use of tensiometers for irrigation purposes has disadvantages such as its high maintenance, its bad performance in dry soil conditions (Muñoz-Carpena et al., 2005) and its limited measurement range (Menne et al., 2022). Granular matrix sensors are an adaptation of the gypsum block ones, since they consist of two electrodes located above a gypsum wafer, embedded within a granular fill matrix. They are simple to use and install, require a low maintenance (Jabro et al., 2020) and are a robust and reliable way to measure SMP (Irmak and Haman, 2001; Nolz et al., 2013; Vellidis et al., 2008). One of the granular matrix sensors commonly used is the Watermark (Irrometer Co. California, USA).

2.1.2. Plant-based water status indicators

Plant itself has long been recognized as the best indicator of its water status. Plant water status can be evaluated by direct measurements of leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potentials or indirectly by measuring leaf gas exchange (stomatal conductance (g_s), net photosynthesis (P_n)), trunk diameter shrinkage, sap flow, leaf turgor or canopy temperature (T_c) (Fernández, 2017).

2.1.2.1. Water potential

Water potential (Ψ) is closely related with plant water status. Traditionally, Ψ is measured with a pressure chamber (Figure 9), according to Turner (1988). This method consists in measuring the hydrostatic pressure (with negative sign) contained in the xylem of the plant (Scholander et al., 1964), so, the more negative the Ψ values are, the worse the plant water status is.

Figure 9. Pressure chamber (model 3000, Soil Moisture Equipment Corp. Santa Barbara, CA, USA).

Ψ varies along the day, depending not only on the meteorological conditions (Suter et al., 2019) (Figure 10), but also according to other factors as leaf position and age (Syvertsen, 1982). In a diurnal time-course some variants of Ψ can be measured: predawn water potential ($\Psi_{predawn}$) and midday leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potential. The $\Psi_{predawn}$ is measured before sunrise when plant and soil water content are in equilibrium (Jones, 2007). Ψ_{leaf} and Ψ_{stem} are measured at midday, when the meteorological conditions are more demanding. The Ψ_{leaf} is measured in sunny leaves without covering, whereas Ψ_{stem} is measured in shaded leaves which are enclosed in an aluminium bag prior to its measure (McCutchan and Shackel, 1992). Between both variants of Ψ, Ψ_{stem} measures are less sensitive to changes in meteorological conditions than Ψ_{leaf} ones.

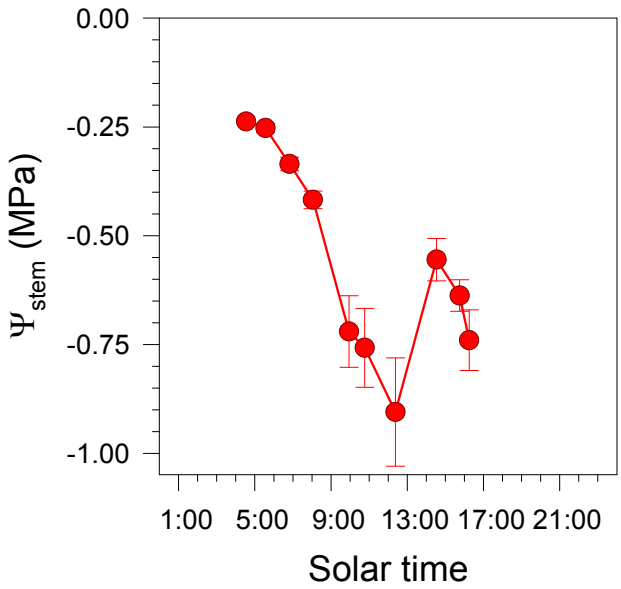

Figure 10. Typical diurnal time-course of stem water potential (Ψ_{stem}) in *Citrus* species.

Nowadays, Ψ is recognized as a robust plant water status indicator for irrigation management purposes (García-Tejera et al., 2021) in several tree crops like olive (Shackel et al., 2021), peach (Mirás-Avalos et al., 2016a) or nectarine (De la Rosa et al., 2013). With this purpose, baselines between Ψ_{stem} and meteorological variables as reference evapotranspiration, solar radiation, vapour pressure deficit and air temperature have been established in several fruit crops (Conejero et al., 2011; Conesa et al., 2016; Ortuño et al., 2006). Besides, different Ψ_{stem} threshold values have been proposed for scheduling irrigation in several crops and their effect on some agronomic traits were also studied. For instance, in peach it was observed that irrigated at Ψ_{stem} threshold values ranged between -1.5 and -1.8 MPa during fruit growth and between -1.5 and -2.0 MPa during postharvest period, being reduced vegetative growth of trees, without affect yield and improving fruit quality (Mirás-Avalos et al., 2016a). Additionally, in mandarin, irrigation scheduling based in the threshold value of Ψ_{stem} of -1.8 MPa during phase II of fruit growth did not affect negatively crop yield (Berríos et al., 2022).

2.1.2.2. Leaf gas exchange

Leaf gas exchange refers to the rate of water vapour going out through stomata, the stomatal conductance (g_s), and the CO_2 entering stomata, the net photosynthesis (P_n). Usually, it is measured using a porometer or a portable photosynthesis system (Figure 11).

Figure 11. Portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE, USA).

The g_s may be affected by changes in the soil water status, in such a way that under optimal soil water conditions stomatal aperture is favoured, while under soil water deficit stomatal closing is promoted (Devi and Reddy, 2020). Indeed, meteorological conditions

also have an influence in g_s measurements (Struthers et al., 2015), showing maximum values early in the morning and in the evening hours, when vapor pressure deficit (VPD) is soft, and minimum values at midday when the VPD is the highest (Figure 12). Additionally, plant hormones like abscisic acid (Castro et al., 2019) and crop phenological stage (Romero-Trigueros et al., 2014) are also involved in the stomatal response.

Leaf g_s is related with leaf P_n, in such a way that stomata regulate CO_2 input, and then, photosynthesis process (Urban et al., 2017). In a diurnal course, P_n, shows a similar trend to g_s (Figure 12).

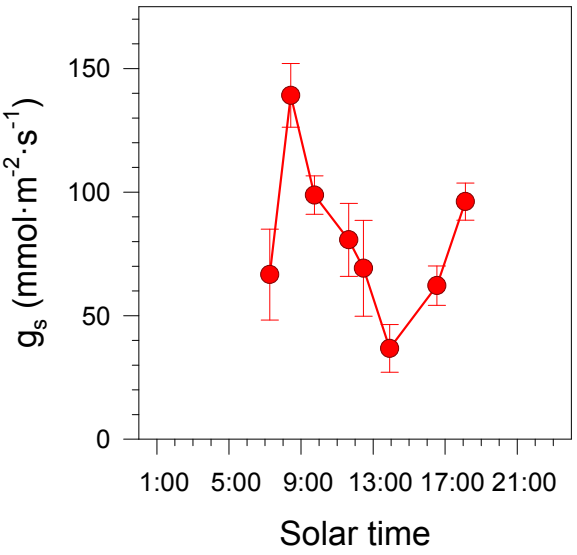

Figure 12. Typical diurnal time-course of stomatal conductance (g_s) in *Citrus* species.

Leaf gas exchange (g_s and P_n) is considered a good plant water status indicator in several woody crops (Rodriguez-Dominguez et al., 2019; Spinelli et al., 2016) and in *Citrus* species (Pérez-Pérez et al., 2009; Romero-Trigueros et al., 2021a). In olive trees, it was reported that it is possible to schedule irrigation from automated estimations of g_s, ensuring an adequate soil water status and avoiding any hard effect on olive yields (Hernandez-Santana et al., 2019). Ramírez et al. (2016) also confirmed that g_s measurements could be appropriate to irrigation scheduling in potatoes. In *Citrus* species, less information exists relative to the use of g_s in the irrigation scheduling. Despite this, optimal g_s values of several *Citrus* species are known (Conesa et al., 2018; Pedrero et al., 2015) and could be used with irrigation management purposes.

2.1.2.3. Canopy temperature

Canopy temperature (T_c) is an indirect measurement of the degree of stomatal aperture (Iseki and Olaleye, 2020; Leinonen et al., 2006) and then, to the plant water status (Jackson, 1982). In such a way that, when stomatal aperture is favoured, an increase in leaf cooling is observed, decreasing its temperature. In contrast, when stomatal closing is promoted, a reduction in leaf refrigeration occurs and consequently an increase in its temperature. In this way, T_c is a good plant water status indicator in Citrus species (González-Dugo et al., 2014; Romero-Trigueros et al., 2019) but also in other crops (Belfiore et al., 2019; Khorsandi et al., 2018).

T_c can be *in-situ* or remotely measured using infrared radiometer sensors (Figure 13) or thermal cameras. This plant water status indicator presents numerous advantages respect to the traditional ones (Ψ and g_s), since it allows us to study a large population of leaves (Bellvert et al., 2016), its measures are easily extrapolated from leaf to field scale (González-Dugo et al., 2014) and its a rapid and non-destructive technique to monitor plant water status (Blaya-Ros et al., 2020).

Figure 13. Infrared radiometer sensors (model SI-431, Apogee Instruments, Inc., Logan, UT, USA).

In a diurnal course, T_c fluctuates with maximum values at midday, when the air temperature (T_a) is the highest, and minimal at the beginning and at the end of the day, coinciding with the lowest values of T_a (Figure 14). To attenuate the effect of meteorological conditions on the absolute values of T_c, several thermal canopy indices have been proposed, as canopy to air temperature difference (T_c-T_a) and the crop water stress index (CWSI) (Idso et al., 1981).

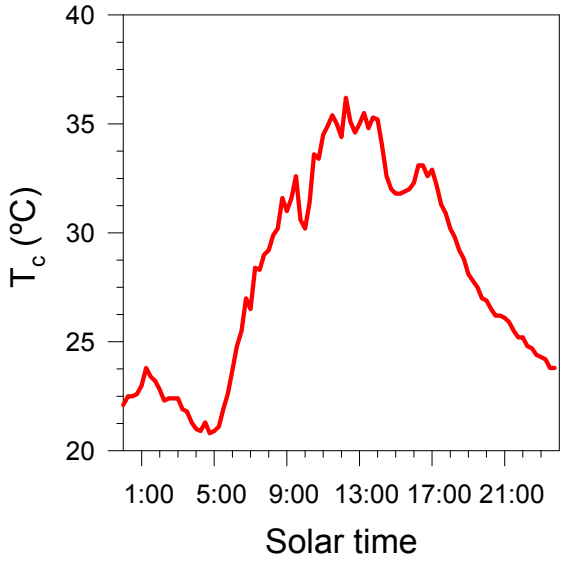

Figure 14. Typical diurnal time-course of canopy temperature (T_c) in *Citrus* species.

CWSI is calculated according to the equation:

$$CWSI = \frac{(T_c - T_a) - (T_c - T_a)LL}{(T_c - T_a)UL - (T_c - T_a)LL} \quad (1)$$

Where, T_c-T_a is the measured canopy to air temperature difference, and (T_c-T_a)LL and (T_c-T_a)UL, correspond with the lower and upper limit of (T_c-T_a), respectively. (T_c-T_a)LL is equivalent to a canopy transpiring at the potential rate. Graphically, it can be represented as a linear relationship between T_c-T_a and VPD for well irrigated trees. (T_c-T_a)UL corresponds to a canopy which transpiration has ceased and can be obtained as the maximum T_c-T_a value recorded in the stressed trees (Yang et al., 2021) or as a value related with T_a (Sepaskhah and Kashefipour, 1994).

One of the potential applications of thermal-based indices in agriculture includes its use in irrigation scheduling (Ishimwe et al., 2014). In this sense, Akkuzu et al. (2013) established in olive trees the CWSI threshold value of 0.4 for an optimal irrigation management. Additionally, Erdem et al. (2005) suggested the CWSI threshold value of 0.6 as the optimal for irrigation initiation in watermelon. In peach, Zhang et al. (2017) established different T_c-T_a thresholds values ranging between -5 to 3 ◦C to schedule irrigation during the post-harvest period. In *Citrus* species, Ballester et al. (2013) observed that values of Ψ_{stem} around -1.0 MPa, indicatives of an absence of plant water stress, correspond with the T_c-T_a threshold value of 1 ◦C.

2.1.2.4. Other plant water status indicators

- Trunk diameter variations

Another indirect plant water status indicator is the trunk diameter variations (TDV). In a daily performance, trunk diameter experiments cycles of shrinkage and dilatation. Thus, during light hours, when climatic conditions are more demanding, water loss by transpiration is higher than root water uptake, resulting in a movement of water phloem to xylem and a trunk shrinkage. On the contrary, at night hours, when climatic conditions are smoother, there is a water movement from xylem to phloem resulting in a trunk dilatation. These micro-variations in the trunk diameter can be continuous and automatically measured using dendrometers. From the TDV measurements several indices can be calculated, one of the most widely used is the maximum daily shrinkage (MDS) proposed by Goldhamer and Fereres (2001). This index is calculated as the difference between the maximum (MXTD) and minimum (MNTD) trunk diameter daily values. The use of MDS for irrigation management has been evaluated with good results in several crops (Fernández and Cuevas, 2010; Mirás-Avalos et al., 2017, 2016b; Ortuño et al., 2010).

- Sap flow

Sap flow is an indicator of plant water needs/status as it can estimate transpiration rate (Lu et al., 2002; Smith and Allen, 1996; Venturin et al., 2020) as the water loss by the plant in a continuous way. Several methods to calculate sap flow exist; one of the most used is the heat-pulse technique (Green and Clothier, 1988). This method consists in emitting a heat pulse, measuring the time it takes to register the same temperature in the probes installed at both sides of the heater. Subsequently, this time delay is used to calculate sap velocity and then, sap flow according to Cohen et al. (1981). This plant water status indicator is proposed as suitable to be used with irrigation scheduling purposes by several authors (González-Altozano et al., 2008; Nicolás et al., 2008; Ortuño et al., 2005).

- Leaf turgor

Leaf turgor also has been proposed as a predictor of plant water status in several woody crops like olive (Padilla-Díaz et al., 2018), clementine (Ballester et al., 2017) and persimmon (Martínez-Gimeno et al., 2017). It is measured with ZIM-probe as the pressure transfer through a patch of an intact leaf, which is inversely correlated with the turgor pressure (Zimmermann et al., 2008). The sensor consists of two stamps equipped

with a magnet. One of the two stamps contains a sensitive pressure sensing-element, which sends the detected pressure changes to the transmitter. The use of the ZIM-probe presents great advantages as it is a non-invasive technique that allows continuous monitoring of the plant water status (Fernández, 2017). These sensors have been used in irrigation management approaches (Barriga et al., 2022; Padilla-Díaz et al., 2016).

2.2. Water use efficiency techniques and strategies

In the current water scarce context, cropping techniques, as shading, or irrigation strategies, like regulated deficit irrigation, have been proposed to increase water use efficiency in Mediterranean areas. Moreover, the use of alternative water sources such as reclaimed water also contributes to mitigate the freshwater shortage problem. The usage of these techniques and strategies could affect plant and soil water status indicators behaviour, so this issue needs to be studied before using these indicators for precise irrigation management.

2.2.1. Shading technique

Shading is a cropping system that protects crops against abiotic (e.g. extreme meteorological conditions: high temperature or radiation) and biotic (e.g. diseases and pest) stresses (Mditshwa et al., 2019). It is performed by covering the trees with a net (Figure 15). The efficiency of the net is influenced by its shading factor, which is a commercial parameter that refers to the ability of it to absorb or reflect solar radiation (Manja and Aoun, 2019). This factor depends on the colour and the material of the net among other characteristics (Castellano et al., 2008). In general, most agricultural shade nets are white (Tinyane et al., 2018) (Figure 15), but black, blue and other colours are also used. Net materials used can be high-density polyethylene (Wachsmann et al., 2014) or aluminate (García-Sánchez et al., 2015) among others.

Figure 15. White high-density polyethylene shade net house with a zig-zag roof.

This cropping technique modifies microclimate surrounding the plants by inducing changes in air temperature, relative humidity, wind speed and the incoming photosynthetically active radiation (PAR). For instance, Alaphilippe et al. (2016) found a decrease in PAR radiation by 15 % in a pear orchard cultivated under Altocarpo-nets. Wachsmann et al. (2014), observed a decrease in the wind speed by 80-90 % in 'Ori' mandarin cultivated under red net. These microclimate modifications affect the plant water status indicators behaviour. Thus, in apple trees grown under photo-selective blue netting, an increase of 28 % in the net photosynthesis was observed (Bastías et al., 2012). In 'Pera' orange, shading induced higher g_s and CO_2 assimilation rates than in open-field conditions (Medina et al., 2002). The use of netting also induces changes in other plant water status indicators such as T_c. Thus, Blakey et al. (2016) observed an increase in T_c in 'Carmen-Hass' avocado trees grown under white netting. In contrast, Jifon and Syvertsen (2003) reported in 'Ruby Red' grapefruit trees cultivated under aluminized screen a decrease in T_c, in comparison with the grown in open-field conditions.

Agronomic traits as vegetative growth of trees can also be affected by the cropping system. In this sense, Iglesias and Alegre (2006) observed an increase in the trunk diameter growth in shaded apple trees. Other vegetative parameters like height also resulted positively affected by this cropping system, as pointed out by García-Sánchez et al. (2015) and Gimeno et al. (2015) in 'Fino 49' lemon trees. In addition, Incesu et al. (2016) reported an increase in the leaf number of 'Lane Late' orange trees growing under shade net.

Productive parameters such as yield and fruit quality, could also be influenced by the cropping system. This statement was confirmed in the avocado crop, in which shaded conditions induced a higher yield than in open-field ones (Tinyane et al., 2018). The positive effect of shading in yield was also reported in *Citrus* species, like 'Ori' mandarin, in which was observed an increase of 17.3 kg per tree under netting (Wachsmann et al., 2014). This cropping system also affects positively external fruit quality traits as fresh weight (Meena et al., 2016) and internal fruit quality parameters as sugar content (total soluble solids) (Otero et al., 2011).

2.2.2. Regulated deficit irrigation

Deficit irrigation is an irrigation strategy, which consists in the application of water below crop evapotranspiration (ET_c) requirements (Fereres and Soriano, 2007). Under the deficit irrigation term, several strategies are included such as regulated deficit irrigation (RDI) and partial root-zone drying (PRD) (Amorim et al., 2021; Ruiz-Sanchez et al., 2010). RDI consists in applying restrictions in the water supply when the sensitivity of plant to water stress is the minimum and fulfil irrigation during the critical phenological periods (Pedrero et al., 2015). This irrigation strategy was firstly applied in Australia by Chalmers et al. (1981) in order to control the vegetative growth of peach trees. Nowadays, this irrigation strategy has been successful applied in many fruit and nut tree species such as almond (Goldhamer and Viveros, 2000), pistachio (Goldhamer and Beede, 2004), citrus (Gasque et al., 2016), apple (Ebel et al., 1995), apricot (Ruiz-Sánchez et al., 2000), olive (Moriana et al., 2003), cherry (Blanco et al., 2019) and peach (Ruiz-Sánchez et al., 2018).

Reductions in the irrigation doses induce modifications in the plant water status. In this sense, deficit irrigation conditions promoted a decrease of Ψ_{stem} values in mandarin trees (Conesa et al., 2018). In grapefruit trees, Romero-Trigueros et al. (2017a) observed that irrigated at 50 % of ET_c during the phase II of fruit growth caused a reduction by 11 % in Ψ_{stem} values respect to the control trees. Besides, lowering in the irrigation dose affects negatively soil water status promoting a decrease in the g_s (Amorim et al., 2021; Pérez-Pérez et al., 2009) and intercellular CO_2 (Miranda et al., 2021), limiting photosynthetic capacity of trees.

Agronomic traits as vegetative growth is soil water availability dependent. In this sense, Ballester et al. (2014) observed a reduction by 19 % in trunk growth rate in RDI

'Clementina de Nules' mandarin trees. In line with this, Pérez-Pérez et al. (2016) reported in lemon trees a decrease in the trunk growth rate by 44 % because of irrigating at 25 % of ET_c along the crop cycle except during the phase II of fruit growth when trees were fully irrigated.

Dealing with crop yield, reductions of 20-40 % of irrigation dose during ripening stages did not significantly affect mandarin yield (Conesa et al., 2018). Similarly, González-Dugo et al. (2018) observed that reductions by 50-55 % n the irrigation doses during summer months (mid-June to early September) had a minimal effect on mandarin and orange yield. In addition, García-Tejero et al. (2010) reported that yield was not statistically different between RDI and control treatments. Respect to fruit quality a positive effect was reported in RDI citrus trees, with an increase in sugars (Conesa et al., 2014), sugar/acid ratio (Romero-Trigueros et al., 2017b) and flavonoid and phenolic content of fruits (Navarro et al., 2015).

2.2.3. Reclaimed water

Reclaimed water (RW) is a promising alternative to ensure water supply in areas with freshwater limitations. In the last few years, RW production has been increasing in the worldwide context (Giakoumis et al., 2020). Spain is the first European country in water recycling and it belongs to the top ten worldwide (Jodar-Abellan et al., 2019). In the national territory, the production of RW is concentrated in the Region of Murcia, Comunidad Valenciana, and Baleares, which recycle 71.8, 47.5 and 33.0 % of total, respectively (INE, 2018). The Region of Murcia is the national recycling water leader with 99 reclaimed water treatment plants and an annual production of 110 hm^3 $year^{-1}$ (CARM, 2022). In this Region most of the water regenerated (approximately 95 %) is used for agricultural purposes.

The use of this water source in agriculture has many advantages associated with its high nutrient content (Romero-Trigueros et al., 2021b), which promotes a soil nutrient enrichment (Gao et al., 2021; Pereira et al., 2012). This fact supposes a potential reduction of the fertilisation rate, and a consequently increasing in farmers´ economic benefits (García-Caparrós et al., 2017). Nevertheless, the application of nutrients in large quantities could contribute to water body's pollution (Thompson et al., 2020), making necessary an appropriate management of this type of water. Besides, the salinity character

of RW, higher than fresh water (Pedrero et al., 2013), should be considered in the irrigation management.

The use of RW with agricultural purposes could influence plant water status. Recently it was reported that irrigate with RW reduces leaf gas exchange (g_s and P_n) and Ψ_{stem} of *Salvia officinalis* consequence of the salinity of this water source (Lorente et al., 2022). The same trend observed Romero-Trigueros et al. (2021a) in grapefruit trees irrigated with RW.

Irrigation water source can also affect agronomic traits as vegetative growth. In this sense, Nicolás et al. (2016) reported a reduction in the vegetative growth of mandarin trees irrigated with RW. In addition, RW treatment also affected nectarine yield, reducing the number of fruits, although this reduction was compensated by an increase in the individual fruit weight (Pedrero et al., 2018). Additionally, in grapefruit trees, fruit quality parameters such as sugar/acid ratio and juice content were increased as a consequence of the irrigation with the saline RW (Romero-Trigueros et al., 2017b). Despite these minor drawbacks, this water source mitigates the freshwater shortage problem (Li et al., 2021) and is an alternative for farmers above desalinated water in terms of profitability and sustainability (López-Serrano et al., 2021).

CHAPTER IV: SCIENTIFIC ARTICLES

ARTICLE I:

Evapotranspiration in young lime trees with automated irrigation

A.B. Mira-García, J. Vera, W. Conejero, M.R. Conesa, M.C. Ruiz-Sánchez

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Objectives

This study was aimed at measuring the evapotranspiration (ET) of potted young lime trees, with water balance method, using a drainage-weighing lysimeter prototype. The novelty of the proposed methodology was that it includes a soil-based irrigation scheduling protocol that calculates the irrigation dose based on threshold values of volumetric soil water content (θ_v).

Materials and Methods

The experiment was carried out at the CEBAS-CSIC experimental field-station in Murcia, Spain. Plant material comprised two-year old Bearss lime trees (*Citrus latifolia* Tan.) grafted on *Citrus macrophylla* L. rootstock. These trees were planted in 45 L polyethylene pots, which contained a clay-loam textured soil. Lime trees were drip irrigated with two emitters per pot (2 L h^{-1} each). The threshold values of θ_v were fixed to 30 % of management allowed depletion and field capacity to trigger and stop irrigation, respectively. Plant water status was assessed on a seasonal and daily basis by discrete measurements of stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials, with a pressure chamber, and net photosynthesis (P_n) and stomatal conductance (g_s), with a portable photosynthesis system.

Lime tree ET was calculated through the generic water balance method, as water gained by irrigation and rainfall and the lost by drainage, once θ_v variations were assumed to be nil. Irrigation, drainage and θ_v variations were real-time monitored by flowmeters, drip gauges and capacitance probes, respectively. In addition, pot weight was measured in a continuous way by a scale. Water balance and plant water relations measures were taken in four drainage-weighing pot-lysimeters, each considered as a replicate.

Results and Discussion

During the experimental period, the lime trees were well irrigated showing midday Ψ_{stem} values close to -0.8 MPa. These Ψ_{stem} values were indicative of non-limiting soil water conditions. A parallel trend was noted for sunny transpiring leaves (Ψ_{leaf}), with mean seasonal values fluctuating around -1.1 MPa. The mean seasonal values of P_n and g_s were ≈ 7 μmol m^{-2} s^{-1} and 80 mmol m^{-2} s^{-1}, respectively, indicative of an optimum plant water status in *Citrus* species.

The diurnal course of Ψ_{stem}, Ψ_{leaf}, g_s and P_n followed a circadian rhythm, according to evaporative demand with maximum values early in the morning and in the afternoon hours and minimum at midday. The fact that their highest values were observed in the afternoon hours, were due to alleviations in ambient conditions. Along the crop cycle the maximum values of these plant water status indicators were observed at harvest. This fact confirmed the positive effect of fruits on plant water relations.

The fortnightly ET values for two-year old lime trees increased from the beginning of the year (starting with 0.25 plant^{-1} day^{-1} in January) to mid-July when the maximum value was recorded (2.56 L plant^{-1} day^{-1}). Subsequently, lime ET gradually decreased until reaching 0.20 L plant^{-1} day^{-1} in December. The high ET values observed in July, during the active lime fruit development stage, were attributed to the elevated water consumption by the crop during the cell expansion process.

Along the experimental period, pot weight showed diurnal cycles decreasing during daylight hours, when the main water consumption of the plants occurred, and remaining almost constant during night hours.

Conclusions

The drainage-weighing lysimeter prototype allowed measuring crop ET throughout the year in a simple, robust and cost-effective way. The automated irrigation protocol based on the threshold values of θ_v allowed to maintain an optimal plant water status. Pot weight served as ET water balance validation, when changes in the crop biomass were negligible. This lysimeter prototype could be replicated to determine accurate ET values in other crops.

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Evapotranspiration in young lime trees with automated irrigation

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ABSTRACT

Citrus latifolia is increasingly being cultivated in Mediterranean areas, where the need for precise irrigation implies a sound knowledge of the crop's water requirements, which is carried out by monitoring the tree water status in the plant-soil-atmosphere system. Two year-old lime trees (*Citrus latifolia* Tan. cv. Bearss) were cultivated in 45 L weighing pot-lysimeters filled with a clay-loam soil and equipped with soil water sensors for recording the real-time soil water status. Irrigation, drainage, pot weight, and agro-meteorological variables were also recorded in real-time. Plant water relations - stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials, and leaf gas exchange - were periodically measured throughout the experiment. The automated irrigation protocol based on the volumetric soil water content was set to prevent tree water stress (with mean seasonal values of Ψ_{stem} above -0.8 MPa and 7 μmol m^{-2} s^{-1} and 80 mmol m^{-2} s^{-1}, for net phtosynthesis and stomatal conductance, respectively). From the soil water pot balance, the computed fortnightly ET mean values throughout the growing season varied from 0.25 to 2.56 L $plant^{-1}$ day^{-1} in winter and summer months, respectively. Maximum ET values occurred in July when evaporative demand was highest and lime fruits were in their most active growing phase. For day length, lime tree biomass changes were negligible, and the pot tree weight variations served for ET water balance validation (r^2=0.92***). The contribution of this work is that it establishes a scalable weighing lysimeter prototype for determining the ET of lime trees grown in Mediterranean semi-arid conditions, using real-time soil water balance monitoring managed by an irrigation automation protocol. This approach could be replicated for precise ET measurements in other crops.

1. Introduction

Lime trees (*Citrus latifolia* Tan.) usually grow under warm/humid climatic conditions near the equator and in subtropical areas of the Mediterranean Basin (Inglese and Sortino, 2019). Globally, lime cultivated area is concentrated in India (286.000 ha), Mexico (175.826 ha) and China (126.804 ha) (FAOSTAT, 2019). In Europe, lime production centres on the Mediterranean regions of Italy and Spain, where the leading lime producer is the Region of Murcia, with 12% of the national production (MAPA, 2019). In this region production has increased by about 18% in the last four years, making this species a potential alternative to traditional *Citrus* cultivars (CREM, 2019).

However, in many areas of the world, water shortages are becoming increasingly common due to periodic droughts, more restrictive laws regulating the use of water for agricultural production, and increased competition for water resources with other non-agricultural uses (Ferères and Soriano, 2007; Fernández-García et al., 2020). Furthermore, climate change is expected to exacerbate this situation in the Mediterranean Basin due to increasing potential evapotranspiration, lower precipitation, and increases in the frequency and intensity of drought (IPCC, 2018). This will be felt most in the agricultural sector, which is the main consumer of water (>70%) (AQUASTAT, 2020). Such a scenario will require new ways to maximize the precision of irrigation so that water is used more efficiently, which, in turn, implies accurately determining the crop water needs.

Crop water requirements are linked with evapotranspiration (ET_c), which integrate crop transpiration and soil evaporation. ET_c is an important component of surface energy and hydrological balances, being responsible for the greatest part of water consumption in agricultural production (Evett et al., 2012). For this reason, an accurate knowledge of crop water requirements (ET_c) is essential for helping growers decide `when´ it is necessary to irrigate a crop and `how much´ water to apply.

There are several indirect methods for calculating crop evapotranspiration (ET_c), such as the FAO-24 Pan evaporation method (Eliades, 1988; Chartzoulakis and Drosos, 1995), and FAO-56 crop coefficient

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method (Allen et al., 1998), which is widely used by the scientific community (Fernández et al., 2010; Xu et al., 2013; Gong et al., 2020; Silva et al., 2020). This method considers climatic variables, which are included in the reference crop evapotranspiration (ET_0), and the crop type and its characteristics, all of which are included in a single crop coefficient (K_c). ET_0 is independent of crop characteristics and can be obtained as a function of meteorological conditions, the Penman-Monteith equation being the most frequently used (Allen et al., 1998). The K_c, however, is intimately dependant on specific crop factors such as the phenological stage, density, pruning, management, variety and rootstock (Doorenbos and Kassam, 1979; Ruiz-Peñalver et al., 2015; Vera-Repullo et al., 2015) . It is also dependant on climate, soil and irrigation conditions, which represents a great disadvantage because it involves making adjustments to reflect local conditions (Abrisqueta et al., 2013).

ET_c can also be accurately determined through direct measurement of the soil water balance using weighing lysimeters (Girona et al., 2011; Evett et al., 2012; López-Urrea et al., 2014; Ruiz-Peñalver et al., 2015; Vera-Repullo et al., 2015; Jiménez-Carvajal et al., 2017), a type of lysimeter consisting of a device which, based on weight differences, accurately measures the inputs and outputs of water flows that affect the soil and tree mass. The soil water balance method is based on the idea that crop evapotranspiration occurs at the expense of the water stored in the root-zone resulting from irrigation, rainfall, and capillary rise (inputs), while discounting runoff and drainage (outputs) (Cohen et al., 1996). This method allows ET_c to be precisely determined, avoiding the imprecision associated with a particular crop, climatic conditions or irrigation factors (Beeson, 2011; López-Urrea et al., 2012, 2014; Ruiz--Canales et al., 2013). In recent years, lysimetric equipment and techniques have improved, enabling the more accurate measurement of water balance parameters (Meissner et al., 2010; Zupanc et al., 2012). For all these reasons, this method is frequently used to validate the ET_c data obtained with other methods (Vaughan et al., 2007; Wegehenkel and Gerke, 2013; Xu et al., 2013) and for the determination of crop coefficient (K_c) values (Allen et al., 1998; Akhand et al., 2014).

On the other hand, given the practical difficulties involved in the precise parameterization of the water balance, soil sensors have been used for empirical site-specific adjustment (Mounzer et al., 2008; Evett et al., 2012; Vera et al., 2019). Thus, the combination of water balance and sensors allows the crop water needs to be accurately calculated. Capacitance-type soil water sensors are widely used in precision irrigation (Paltineanu and Starr, 1997; Abrisqueta et al., 2012; Vera et al., 2013, 2019, 2021). They function by determining the dielectric permittivity of the soil around the sensor, which mostly depends on the volumetric soil water content (θ_v).

This study is aimed at measuring the evapotranspiration of young lime trees (ET) during the growing season in Mediterranean conditions, using the water balance method. Two-year old lime trees were planted in pot-weighing lysimeters equipped with sensors for the real-time monitoring of irrigation, drainage, pot weight, θ_v. Plant water relations were assessed by measuring stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials, as well as leaf gas exchange (net photosynthesis and stomatal conductance) on a seasonal and diurnal basis. The novelty of the proposed methodology is that it includes a soil-based irrigation scheduling protocol that calculates the correct irrigation dose based on a feed-back strategy. This strategy involves replenishing the water used by the plants based on changes in θ_v as a control system variable.

2. Materials and methods

2.1. Experimental conditions

The experiment was carried in a lime tree orchard located at the CEBAS-CSIC experimental field-station in Murcia, Spain (38° 06′ 31′' N, 1° 02′ 14′'W) at 110 m.a.s.l. The plant material consisted of two-year old lime trees (*Citrus latifolia* Tan., cv. Bearss) grafted on *Citrus macrophylla* L. rootstock.

Fifteen trees were planted in 45 L polyethylene pots (410 mm diameter and 420 mm height), containing a highly calcareous soil (45% calcium carbonate), with a clay loam texture, low organic matter content and bulk density of 1.43 g cm^{-3}. Soil water characteristics were obtained using the theoretical Saxton model (Saxton et al., 1986). The soil water content at field capacity (FC) and permanent wilting point (WP) were 0.35 and 0.15 m^3 m^{-3}, respectively. Pots were located in open-air conditions, and painted white to prevent the roots from overheating in the sun.

The trees were drip irrigated with two self-compensating emitters (2 L h^{-1} each) per pot, placed at two points to ensure uniform water distribution at the pot surface.

Lime trees showed homogenous vegetative development: tree height of 1.01 ± 0.06 m, mean trunk diameter (0.03 m above soil surface) of 23.7 ± 1.88 mm, total leaf area of 0.55 ± 0.01 m^2 (≈ 155 leaves per tree, 35.5 cm^2 $leaf^{-1}$), and a tree canopy cover of 0.26 m^2.

2.2. Irrigation protocol

An automated irrigation scheduling protocol was applied, using the volumetric soil water content (θ_v) as a control system variable. For this purpose, four pots containing trees of similar appearance were selected and equipped with capacitance probes (one per pot) (EnviroPro EP100G-04, Entelechy Pty Ltd., St. Agnes, Australia), for making continuous θ_v and soil temperature measurements. The capacitance probes were calibrated for a clay-loam textured soil following the procedure proposed by Paltineanu and Starr (1997). Each probe was installed midway between tree trunk and pot edge, and connected to a radio transmission unit, which read the values every 5 min and recorded average values every 15 min. Only sensors located at 0.20 and 0.30 m depth were read. The radio-transmission units sent data to a gateway connected TCP/IP to the addVANTAGE cloud-based platform (ADCON Telemetry, Vienna, Austria) for data processing and visualization. Maximum and minimum θ_v threshold values were fixed in such a way that the feed-back system control remotely acted on the electro-hydraulic valve, triggering or stopping the irrigation.

At the beginning of the experimental period, irrigation threshold θv values were tested in order to provide an adequate plant water status and no soil anoxia, avoiding tree water stress. θ_v values at 0.2 and 0.3 m depth were averaged to calculate threshold values. As a result, the threshold θ_v values were fixed to 30% of MAD (management allowed depletion) and field capacity (FC) to trigger and stop irrigation, respectively. MAD was calculated from the following equation (Merriam, 1966; Allen et al., 1998):

$$MAD = \alpha\, AWC \quad (1)$$

where α is a dimensionless coefficient (equal to 0.3), and AWC is the available soil water content, calculated as: AWC = FC – WP.

2.3. Measurements

2.3.1. Environmental conditions

Agro-meteorological data (air temperature, relative humidity, solar radiation, wind velocity and rainfall) were recorded by an automated weather station (http://www.cebas.csic.es/general_spain/est_meteo.html) located in the CEBAS-CSIC experimental field station, which read the values every 5 min and recorded the averages every 15 min. From these data, reference crop evapotranspiration (ET_0) values were calculated hourly using the Penman-Monteith equation (Allen et al., 1998). Vapour pressure deficit (VPD) was calculated from air temperature and relative humidity values (Allen et al., 1998).

2.3.2. ET measurement and pot-lysimeters

Lime tree evapotranspiration (ET) was calculated using the generic

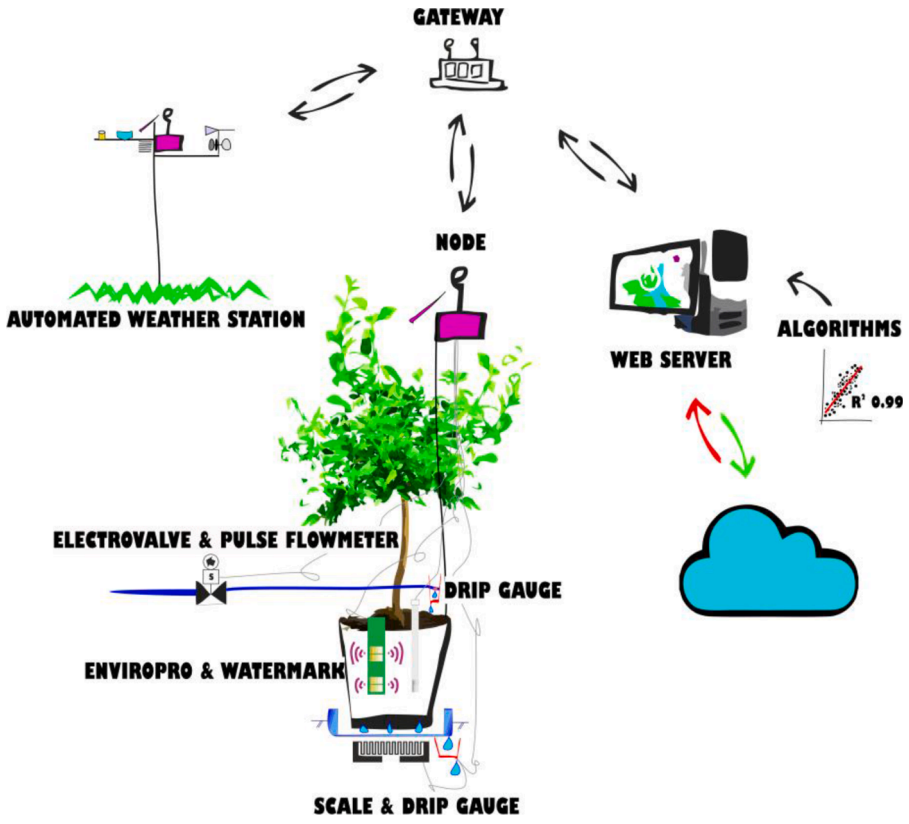

Fig. 1. Design of the weighing pot-lysimeter, including sensors and telemetry network, for evapotranspiration (ET) measurements of young lime tree (by W. Conejero).

water balance equation:

$$ET = I + R - RO - D \pm \Delta SW \quad (2)$$

Under the experimental conditions, the runoff (RO) was zero, except for the heavy rain episode (82.4 mm in March). Additionally, under high frequency irrigation and the automated irrigation protocol applied, the soil water content variations (ΔSW) were negligible and then ET could be calculated as water gained by irrigation (I) and rainfall (R) and water lost by drainage (D). Hence, Eq. (2) becomes:

$$ET = I + R - D \quad (3)$$

The four lime tree pots were arranged as to fit in weighing pot-lysimeters (Fig. 1). The amount of water applied through irrigation (I) was measured with a pulse flowmeter (Sensus, 120 HRI-A, Barcelona, Spain) located at the beginning of the fifteen pots. Also, drip gauges (Pronamic, Ringkoebing, Denmark) were installed below the emitter near the capacitance probe to monitor real-time irrigation amounts and to detect any flow rate failures during the irrigation events. Drainage (D) was measured with drip gauges (Pronamic ApS, Ringkoebing, Denmark) located under each pot. ΔSW was monitored with capacitance probes (as described in Section 2.2.).

The four selected lime tree pots were placed on four calibrated scales (150 kg load cell ± 20 g, ZMISSIL F2–150, Gram Precision, Spain) to determine the pot weight (W), which, together with the water balance parameters, were integrated into the telemetry platform (Fig. 1).

The contribution of soil evaporation (E) to ET was evaluated by covering the soil surface with white plastic of two out the four lime tree pots during several rain-free cycles (each seven-days long) along the experimental period. The difference in ET values between covered and uncovered pot-lysimeters, as determined using Eq. (3), was attributed to E.

2.3.3. Plant water relations

Lime tree water relations were evaluated by discrete measurements of stem (Ψ_{stem}) and leaf (Ψ_{leaf}) water potentials, and leaf gas exchange parameters. On representative, clear days of the growing season, Ψ_{leaf} and Ψ_{stem} were measured around solar noon (12:00 h solar time) using a pressure chamber (Soil Moisture Equipment Corp., Model 3000, Santa Barbara, CA, USA) in mature leaves located near the trunk on the north face of the trees. Ψ_{leaf} was measured on fully expanded (3 to 5 months old), sun-exposed uncovered leaves, following the recommendations described in Hsiao (1990), while for Ψ_{stem} measurements the leaves were covered with aluminium foil at least 2 h before excision (McCutchan and Shackel, 1992).

Leaf gas exchange parameters - net photosynthesis (P_n, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) - were measured in the morning (≈ 8:00 h solar time) the same days as Ψ_{leaf}, on sunny leaves using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a broad leaf chamber (6 cm^2). Measurements were taken in near constant ambient conditions: CO_2 concentration ≈ 400 μmol mol^{-1} and light intensity ≈ 1200 μmol m^{-2} s^{-1}. The air flow rate inside the chamber was maintained at ≈ 350 μmol s^{-1}.

In addition, several diurnal cycles of Ψ_{stem}, Ψ_{leaf} and leaf gas exchange parameters were determined on representative days of the main *Citrus* phenological stages: flowering, stage I and II of fruit growth, and harvest. Measurements were taken from pre-dawn to early afternoon at 0.5 - 1.5 h intervals.

Water balance and plant water relations measurements were taken in the four weighing pot-lysimeters, each one considered as a replicate (n = 4). The lysimeters were placed at the centre of the experimental orchard, and the remaining 11 border lime trees were placed around them according to standard tree spacing (5 m x 6 m).

Linear and nonlinear regression analysis was applied to determine the relationships between plant water relations and environmental parameters using SigmaPlot 13.0 software (Systat Software, San Jose, CA,

Table 1
Monthly values of total crop reference evapotranspiration (ET_0); maximum, mean and minimum daily air temperatures (T_{max}, T_{mean} and T_{min}); maximum, mean and minimum relative humidity (RH_{max}, RH_{mean} and RH_{min}); mean vapour pressure deficit (VPD); total rainfall; mean wind speed at 2 m (u_2) and solar radiation.

Month	ET_0 (mm)	Temperature (°C)			RH (%)			VPD (kPa)	Rainfall (mm)	u_2 (m s^{-1})	Solar radiation (W m^{-2})
		T_{max}	T_{mean}	T_{min}	RH_{max}	RH_{mean}	RH_{min}				
January	42.8	15.5	10.3	6.1	84.0	65.6	47.0	0.98	74.6	8.3	103.5
February	55.0	18.7	13.3	9.0	83.5	65.4	44.4	1.22	3.6	9.4	129.7
March	89.1	21.9	15.2	9.6	86.8	61.7	35.7	1.80	26.4	9.8	192.1
April	95.0	22.5	16.3	10.8	88.9	64.9	39.3	1.76	1.8	6.7	226.9
May	140.4	27.0	20.7	14.6	82.7	56.4	32.3	2.45	0.0	6.6	304.6
June	164.5	31.7	25.5	19.3	82.5	55.4	29.5	3.36	0.0	7.6	332.1
July	158.7	32.3	26.6	21.0	88.8	62.1	37.5	3.09	3.8	7.5	306.3
August	123.0	31.9	26.2	21.4	91.2	69.9	42.1	2.83	8.0	7.5	240.7
September	96.5	29.4	23.2	18.3	92.0	69.2	41.1	2.49	14.6	7.2	228.6
October	71.3	26.6	20.1	15.4	93.7	74.4	46.3	1.91	5.2	9.3	151.6
November	40.5	20.9	14.5	9.7	82.9	59.7	37.6	1.59	6.7	6.3	96.6
December	35.6	17.4	11.6	7.0	77.7	58.1	39.1	1.23	0.6	7.9	96.1
Total/Mean	**1112.3**	**24.6**	**18.7**	**13.6**	**86.2**	**63.6**	**39.3**	**2.06**	**145.3**	**7.8**	**200.7**

USA).

3. Results and discussion

3.1. Meteorological variables

Agro-meteorological data for the 2017 growing season are shown in Table 1. The weather was typically Mediterranean: hot, dry summer and mild, wet winter. Total annual rainfall and ET_0 amounted 145.3 and 1112.3 mm, respectively, which, considering the 10-year seasonal average data for the studied area (rainfall and ET_0 of 250 and 1320 mm, respectively) denoted a dry year (AEMET, 2020).

Monthly ET_0 was the highest in June, coinciding with the longest days and January was the wettest month. Annual daily average maximum, mean and minimum temperatures were 24.68, 18.67 and 13.54 °C, respectively. In agreement with the evaporative demand, the highest temperatures were recorded in summer, with a maximum air temperatures during August, whereas the lowest temperatures were recorded in winter (especially in January). Mean VPD ranged from 3.36 kPa in June to 0.98 kPa in January. Lastly, mean wind speed and solar radiation registered their highest values in March and June, respectively. October was also characterized as a windy month (Table 1).

3.2. Soil water status and automated control of irrigation

The volumetric soil water content differed slightly between the four pots with similar trends at the two depths (0.2 and 0.3 m) throughout the studied season (data not shown). The θ_v at both depths were averaged, representing the full soil profile.

Rainfall events were integrated in the automated irrigation protocol as increases in the soil water content. For example, the rainfall event on 12th −14th March (82.4 mm) caused a rapid increase in the soil water content, with values exceeding saturation values ($\theta_v \approx 45\%$). As a result, irrigation was delayed for six days (Fig. 3).

Data in Fig. 3 also revealed that θ_v varied not only as a consequence of irrigation or rainfall events, but also fluctuated following diurnal air temperature and root water uptake dynamics. These fluctuations were closely related to the evapotranspiration demand, confirming the sensitivity of this type of capacitance water content sensor to the nearby environment of soil and plant roots (Vera et al., 2017, 2021).

The pot weight (W) offered sound information on water dynamics in the whole plant-soil system, the values decreasing during daylight hours when leaf stomata open and the plant water consumption, and remaining fairly constant during the night (Fig. 3). The pot weight integrated both tree biomass and soil water content changes, revealing itself to be less stable than θ_v over periods of time longer than one week (Fig. 2C). For this reason, if pot weight is used as a control system variable for automatic irrigation management, the threshold values would need to be adjusted as soon as the plant biomass changed.

3.3. Plant water relations

During the experimental period, the lime trees were well irrigated to show midday Ψ_{stem} values close to −0.8 MPa, ranging from −1.0 MPa during the fruit growth stages to −0.4 MPa early in the season at the flowering stage (Fig. 4B). A parallel trend with lower values was noted for sunny transpiring leaves, with mean seasonal Ψ_{leaf} values fluctuating around −1.1 MPa (Fig. 4B). The lowest values were measured during the summer months in response to increased evaporative demand, when maximum VPD values were recorded (Fig. 4A). These Ψ_{stem} values were indicative of non-limiting soil water conditions, as widely reported for other *Citrus* species, such as mandarin (González Altozano and Castel, 2003; Ballester et al., 2011; Pagán et al., 2012; Conesa et al., 2014, 2018), orange (Pérez-Pérez et al., 2009; García-Tejero et al., 2010) lemon (Domingo et al., 1996; Ortuño et al., 2005), and grapefruit (Pérez-Pérez et al., 2014; Romero-Trigueros et al., 2017). In this sense, González-Altozano and Castel (1999) established a threshold range for Ψ_{leaf} values of −1.3 to −1.5 MPa to avoid reductions in mandarin fruit size, similarly to that observed in grapefruit (Cohen and Goell, 1988).

The mean seasonal values for leaf gas exchange were ≈ 7 μmol m^{-2} s^{-1} and 80 mmol m^{-2} s^{-1}, for P_n and g_s, respectively (Fig. 4C), with more pronounced variations than in the case of Ψ_{stem} and Ψ_{leaf} values (Fig. 4B). Although the mean values registered in our experiment were slightly lower than those reported under field-grown non-limiting soil water conditions, the same seasonal leaf gas exchange trend has also been observed in orange (Moreshet and Green, 1984; Pérez-Pérez et al., 2008) and lemon trees (Domingo et al., 1996; Alarcón et al., 2006; Ortuño et al., 2006).

Generally, the values of both leaf gas exchange parameters increased from the beginning of the season onwards, as leaves aged, with maximum values P_n of ≈ 11 μmol m^{-2} s^{-1} at harvest, after which the photosynthetic activity tended to decrease (Fig. 4C). The higher P_n values during the active fruit growth stages in lime can be attributed to a feedback effect of the fruit load on leaf photosynthesis (Ben Mimoun et al., 1996; García-Orellana et al., 2013). Furthermore, the good linear relationship found between P_n and g_s [$P_n = 52.01\ g_s + 2.76\ g_s$; $r^2 = 0.66$, $p < 0.001$] (data not shown) demonstrated the clear influence of stomatal behaviour on leaf photosynthetic activity (Chaves et al., 1987).

In an attempt to deepen our knowledge of plant water relations in young potted lime trees under automated irrigation, the diurnal courses of plant water status parameters were evaluated during the growing season. Ψ_{stem} and Ψ_{leaf} followed a circadian rhythm, following changes in the evaporative demand (Fig. 5A), with the highest values recorded predawn (early morning) and the lowest around midday (Fig. 5B), as is

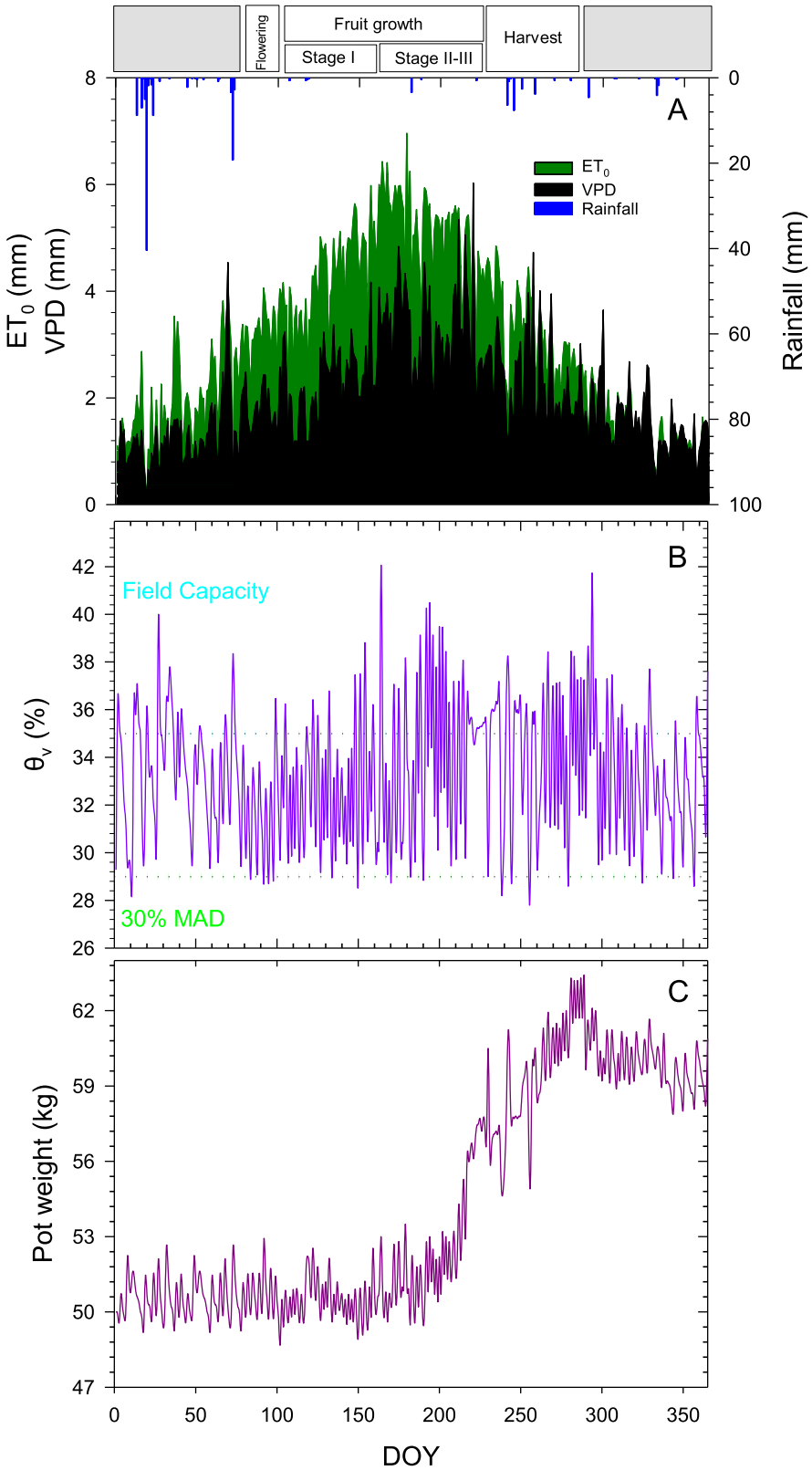

Fig. 2. Seasonal trend of: **A)** Daily crop reference evapotranspiration (ET_0,), vapour pressure deficit (VPD,), and rainfall ( ); **B)** Volumetric soil water content (θ_v, —); and **C)** Pot weight (—) of young lime trees during 2017. DOY: day of year. Lines in B and C are 15-min means of 4 replications. Horizontal dotted lines in B are the θ_v threshold values for automatic irrigation management: FC (field capacity) and 30% MAD (management allowed depletion). Main phenological periods of the lime trees are depicted in the top box.

Table 2
Fortnightly evapotranspiration (ET) and main phenological growth stages of two-year old lime trees growing in Mediterranean climate.

Date	ET (L plant^{-1} day^{-1})	Phenological stage	BBCH scale*
Jan. 1–15	0.30 ± 0.04		
Jan. 16–31	0.31 ± 0.08		
Feb. 1–15	0.25 ± 0.04	Floral induction	*51*
Feb. 16–28	0.40 ± 0.04	Bud break	*53*
Mar. 1–15	0.71 ± 0.07	Flowering	*61*
Mar. 16–31	0.66 ± 0.15		
Apr- 1–15	0.53 ± 0.09		
Apr. 16–30	0.95 ± 0.14	Stage I fruit growth	*67*
May 1–15	0.97 ± 0.08		
May 16–31	1.12 ± 0.20		
June 1–15	1.04 ± 0.13		
June 16–30	1.60 ± 0.17		
July 1–15	1.91 ± 0.24	Stage II fruit growth	*74–79*
July 16–31	2.03 ± 0.20		
Aug. 1–15	2.28 ± 0.10		
Aug. 16–31	2.82 ± 0.20	Main harvest	*89*
Sept. 1–15	2.50 ± 0.19		
Sept. 16–30	1.92 ± 0.30		
Oct. 1–15	1.54 ± 0.08		
Oct. 16–31	1.41 ± 0.12	Second harvest	
Nov. 1–15	1.06 ± 0.11		
Nov. 16–30	0.64 ± 0.03		
Dec. 1–15	0.40 ± 0.03		
Dec. 16–31	0.31 ± 0.01		

*from Agustí et al. (2003) *51*: Transition of resting buds to floral buds; *53*: Bud break and inflorescence development, *61*: Flowering and initial fruit set, *67*: Fruit cell division and physiological fruit drop, *74–79*: fruit cell expansion, 89: fruit suitable for consumption.

typical for most cultivated plants including *Citrus* (Sánchez-blanco et al., 1990; Ruiz-Sánchez et al., 1997, 2007). Those values recorded for sunny freely transpiring non-covered leaves (Ψ_{leaf}) were lower than those for Ψ_{stem} (Fig. 5B). The similar values recorded for these two parameters predawn reflected a lack of transpiration at that time of day. However, as the season progressed, the difference between Ψ_{stem} and Ψ_{leaf} values ($\Delta\Psi$) became more pronounced, and the highest values were recorded during the last diurnal cycle studied (Fig. 5 B), coinciding with harvest and confirming the above mentioned positive effect of fruits on leaf transpiration and photosynthesis processes. $\Delta\Psi$ values have been closely related with daily tree transpiration in lemon (Ortuño et al., 2006; García-Orellana et al., 2013) and almond (Torrecillas et al., 1989) trees.

In a companion paper of Mira-García et al. (2020), the authors reported a strong dependence of Ψ_{stem} on VPD in field-grown lime trees. In lemon trees, García-Orellana et al. (2013) found a higher coefficient of determination in the Ψ_{leaf} *vs.* VPD relationship than for Ψ_{stem} *vs.* VPD, underlining the greater dependence of Ψ_{leaf} on the prevailing atmospheric conditions at the time that measurements are taken (Rodrigues et al., 2012). Indeed, Ψ_{leaf} responds not only to soil water status but also to other factors, including cultivar, environment, soil type and the relationships between canopy and root system (García-Tejera et al., 2021). In this sense, Remorini and Massai (2003) suggested that the Ψ_{stem} value at noon is a better indicator of plant water status than Ψ_{leaf} in peach trees. Moreover, Abrisqueta et al. (2015), Conesa et al. (2019) and Vera et al. (2013) indicated that Ψ_{stem} has long been accepted as the best reference plant water status for early-maturing *Prunus* trees. Nevertheless, in our experimental conditions, both methods of estimating lime tree water status were closely correlated [$\Psi_{stem} = 0.92\ (\Psi_{leaf})^2 + 2.56\ \Psi_{leaf} + 0.30$; $r^2 = 0.79$, $p < 0.001$], as Williams and Araujo (2002) found in vineyards, in which both plant water status indicators were also significantly correlated with θ_v.

The circadian pattern observed for g_s and P_n were characterised by maximal stomatal opening and greatest photosynthetic activity early in the morning as soon as solar radiation reached the plant leaves, followed by a continuously decreasing tendency with minimal values reached by midday (Fig. 5C). As in most *Citrus* sp., this stomatal closure occurred in response to the increasing evaporative demand and the rising air temperatures. (Sun and Ma, 1999) indicated that the best temperatures for optimal crop development of *Citrus* trees varied between 22 and 30 °C in order to avoid stomatal closure. Several studies have pointed to a slight increase in gas exchange in *Citrus* leaves during afternoon hours, probably as a consequence of alleviations in ambient conditions (Sánchez-Blanco et al., 1990; Nicolás et al., 2008; Mira-García et al., 2020).

3.4. ET in young lime trees

Having confirmed that the automated irrigation protocol generated an adequate plant water status in young potted lime trees, ET-water balance computations were carried out following Eq. (3). Although the data were analysed on a weekly basis, for practical purposes fortnightly ET values are presented.

First, it is important to note that ET includes water losses resulting from soil evaporation (E) and from plant transpiration through the stomata (T). Evaluation of the ET in covered and uncovered pot-lysimeters showed that E accounted for a maximum of 20% of ET in the summer months (data not shown). The soil evaporation process (E) depends on temperature (Kerridge et al., 2013), so that increases in soil temperature lower the θ_v and increase the evaporation rate (Kidron and Kronenfeld, 2015). Soil temperature in the potted lime trees varied from 13 to 27 °C in winter and summer months, respectively, which indicated

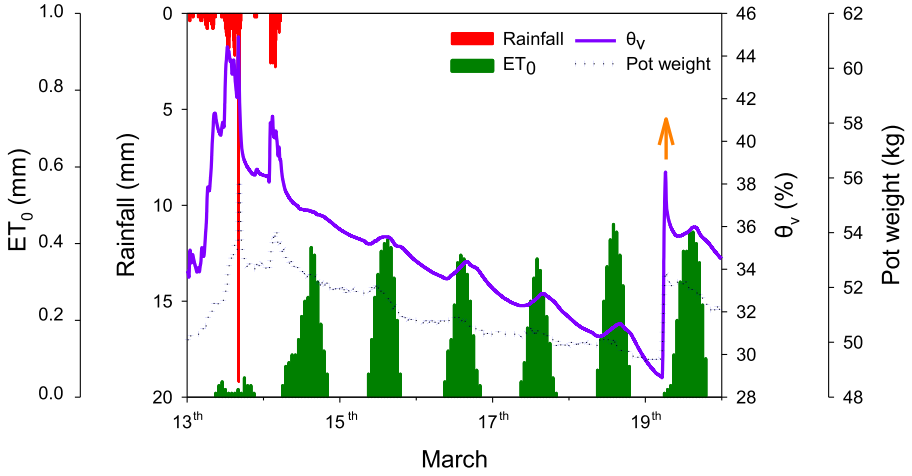

Fig. 3. Hourly crop reference evapotranspiration (ET_0,), 15-min rainfall ( ), volumetric soil water content (θ_v,), and pot weight ( ) of young lime trees during 13th - 20th March 2017. θ_v and pot weight values are 15-min means of 4 replications. Irrigation event is denoted with the orange arrow.

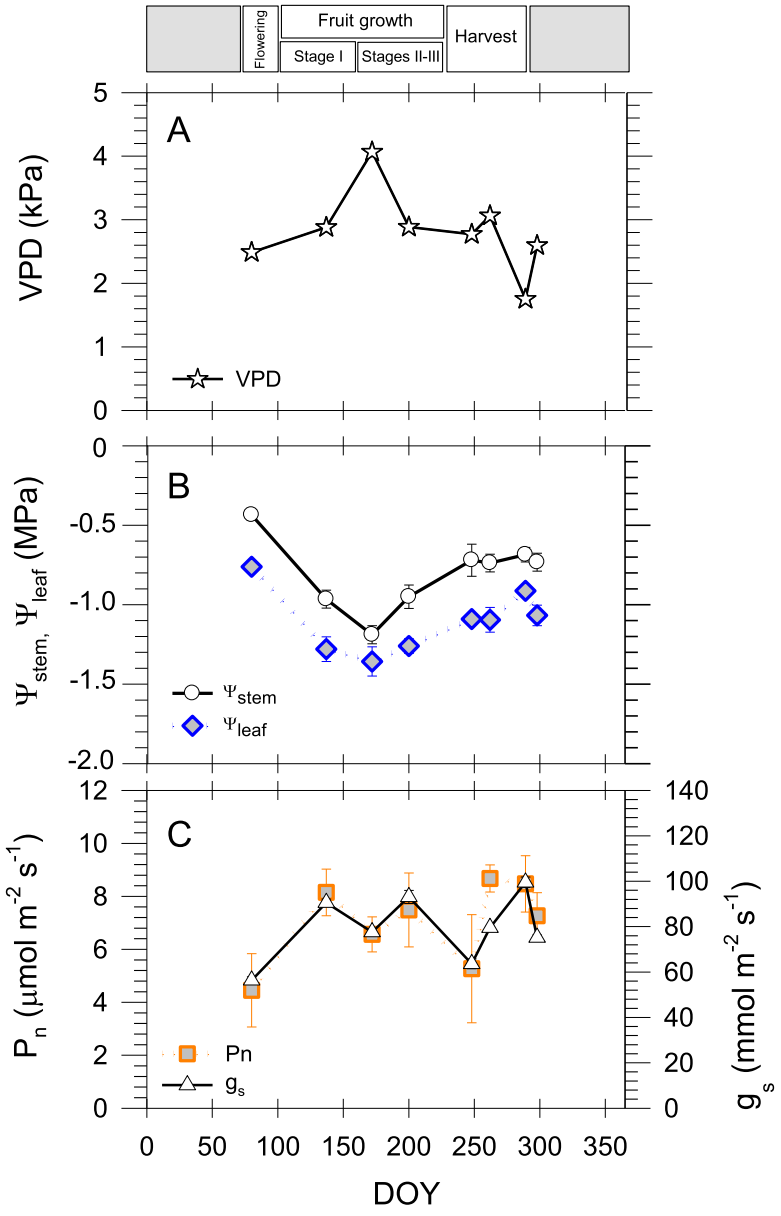

Fig. 4. Seasonal trend of: **(A)** Daily vapour pressure deficit (VPD, ☆), **(B)** Stem (Ψ_{stem}, ○) and leaf (Ψ_{leaf}, ◇) water potentials; and **(C)** net photosynthesis (P_n, □) and stomatal conductance (g_s, △) in young lime trees during 2017. DOY: day of year. Values are means of 4 replications. Vertical bars on data points are ± standard error (SE). Main phenological periods for lime trees are depicted in the top box.

that contribution of E to ET under the experimental conditions was low. However, in field grown *Citrus* sp., Castel (1997) found that soil evaporation accounted for about 8–30% ET in periods with little or no rain and as much as 50% when rain was more frequent and abundant. In cereal crops, the contribution of E to ET decreased from 80% at the beginning cultivation to 5% at the flag leaf stage (Silva et al., 2020).

The fortnightly evapotranspiration (ET) values obtained in the pot-lysimeters for two-year old lime trees grown under Mediterranean climatic conditions with a clay-loamy soil, along with the main phenological stages and their corresponding Biologische Bundesanstalt, Bundessortenamt und CHemische industrie (BBCH) scale for *Citrus* sp. by Agustí et al. (2003), are shown in Table 2. It can be seen that ET gradually increased from the beginning of the year (starting with 0.25 L plant^{-1} day^{-1} in January) to mid-July when maximum values (2.56 L plant^{-1} day^{-1}) were reached. Subsequently, ET gradually decreased until reaching 0.20 L plant^{-1} day^{-1} in December (Table 2).

The same pattern has been also found in other studies in *Citrus* sp. For example, in young (3–4 years old) sweet orange trees grown under surface irrigation, Castel et al. (1987) reported evapotranspiration values (measured by the water balance method) that varied from a minimum of 0.5 - 1 mm day^{-1} to a maximum of 3 mm day^{-1} for the coldest and hottest months of the year, respectively. In eight-year old orange trees grown in lysimeters, Yang et al. (2003) also observed minimum ET values in winter (0.22 mm day^{-1}) and maximum values in summer (4.4 mm day^{-1}). Using the eddy covariance method in mandarin, Maestre-Valero et al. (2017) reported maximum ET values in summer months (about 4.3 mm day^{-1}), and minimum values at the beginning and at the end of the year (≈ 0.11 mm day^{-1}).

In drip irrigated Tahiti lime trees, Alves et al. (2007) reported maximum ET values in December (summer at a Brazilian latitude), as measured in a weighing lysimeter. The authors found that ET varied from 1.3 to 5 mm day^{-1} during the three-year study. Since the

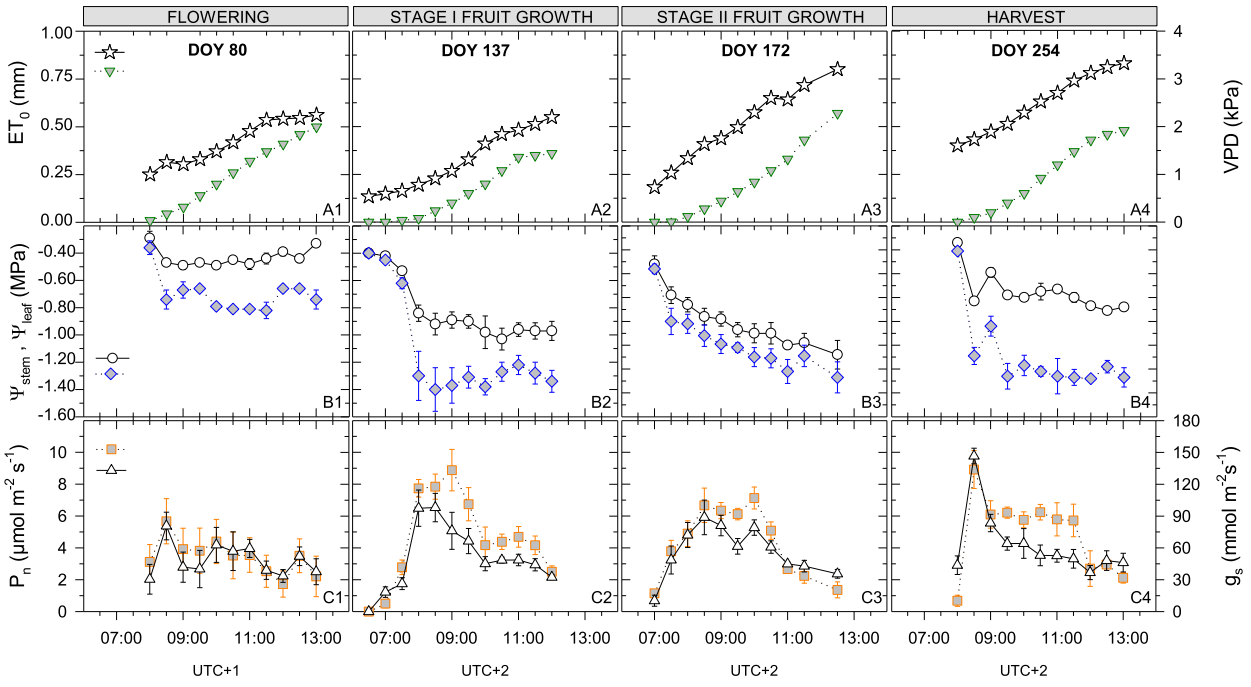

Fig. 5. Diurnal courses of: **(A)** crop reference evapotranspiration (ET_0, ···▽···), vapour pressure deficit (VPD, -☆-); **(B)** Stem (Ψ_{stem}, -○-) and leaf (Ψ_{leaf}, ◇) water potentials; and **(C)** net photosynthesis (P_n, □) and stomatal conductance (g_s, Δ) on (1): 21st March (DOY 80), (2): 17th May (DOY 137), (3): 21st June and (DOY 172) (4): 11th September 2017 (DOY 254). Values are means of 4 replications. Vertical bars on data points are ± standard error (SE). Phenological periods for the lime trees are depicted in the top box. UTC: Universal time coordinated.

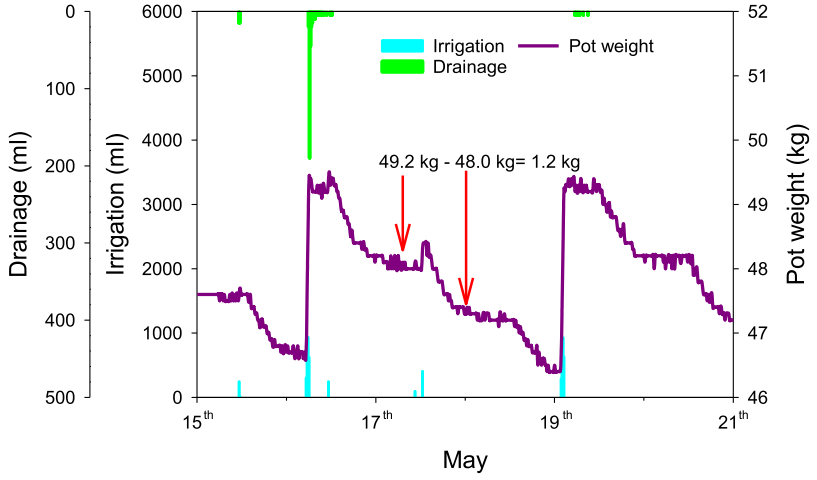

Fig. 6. Irrigation (I, ▬), drainage (D, ▬), and pot weight (—) in young lime trees during 15th −21st May 2017. Values are 15-min means of 4 replications.

evaporative demand conditions in this study were quite similar to our experimental conditions, the slightly higher values than those obtained for Bearss lime trees in our study (Table 2) could be attributed to the increased transpiration as a consequence of the greater canopy development of field-grown Tahiti lime trees.

Apart from the dependence of ET on meteorological parameters, as also seen in other tree crops (Yang et al., 2003; Girona et al., 2004; López-Urrea et al., 2012; Alberto et al., 2014; Maestre-Valero et al., 2017; Peddinti et al., 2020), its relation with the crop phenological stage was also evident. In lime trees ET gradually increased from flowering (0.39 L plant^{-1} day^{-1}) to stage II of fruit growth (2.50 L plant^{-1} day^{-1}), and decreased after the harvesting period, which included two pickings, with minimum ET values reached during the inactive vegetative growth period (0.2 L plant^{-1} day^{-1}) (Table 2). The higher ET values observed during the active fruit development stage were attributed to high water consumption in the cell division and expansion processes (Peddinti et al., 2020).

The pot weight, which remained constant at night and decreased during daylight hours, when plants consumed water (Fig. 3), was considered a more global indicator in the water balance approach. For this reason, it can be used for ET measurements of young lime trees, as long as biomass changes are negligible (Fig. 2C). Data measured for one week in May (Fig. 6) showed that mean pot weight decreased from 49.2 to 48.0 kg in two consecutive days (17th - 18th May), generating a

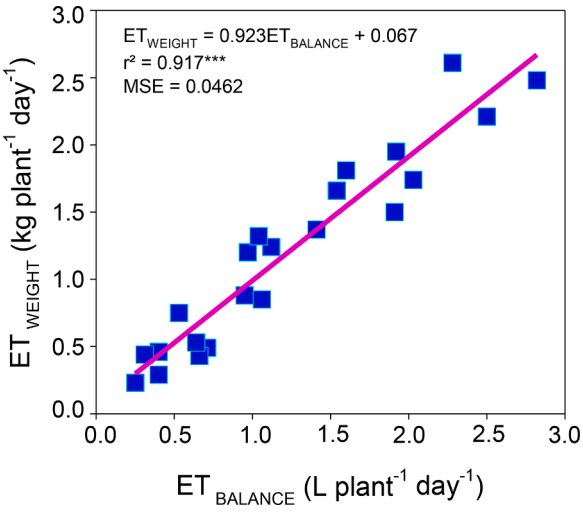

Fig. 7. Relationship between ET of two-year old lime trees calculated as potted tree weight variations (ET_{WEIGHT}) and as soil water balance ($ET_{BALANCE}$). Each point is the mean of 4 replications; ***: $P < 0.001$, MSE: mean squared error.

similar change (1.2 kg) to that measured by the water balance (Eq. (3)) that week: the cumulative amount of irrigation water applied was 8915 ml and the drainage was 435 ml (Fig. 6), in other words 1.2 L plant^{-1} day^{-1}. This value is included in the mean ET value proposed for the second fortnight of May (Table 2). The pot weight therefore served for ET validation, as evidenced by the close relationship found between the two methods of calculating ET: soil water balance ($ET_{BALANCE}$) and pot tree weight variations (ET_{WEIGHT}) (Fig. 7).

4. Conclusions

Under Mediterranean conditions and without water limitation, fortnightly ET values of two-year-old lime trees (*Citrus latifolia* Tan. cv. Bearss) on *C. macrophylla* rootstocks grown in 45 L pots ranged from 0.25 to 2.56 litres per plant and day.

These ET values were obtained with an automated irrigation protocol based on the 30% MAD criteria, using volumetric soil water sensors, and checking the plant water status, with optimal values of stem/leaf water potentials and net photosynthesis of the lime trees.

A prototype of a simple and robust scalable weighing lysimeter was set up for young potted lime trees, with real-time monitoring of irrigation parameters and soil water balance, providing a practical and cost-effective way to measure ET throughout the year.

The proposed automated irrigation approach allowed accurate ET values to be obtained. Assuming a negligible change in tree biomass for day-length periods, the pot weight which remained constant at night and decreased during daylight hours when plant water consumption occurred, served as a validation of ET.

This approach -a combination of pot-lysimeter, automated MAD protocol and soil-plant water status monitoring- could be replicated to determine precise ET values for other crops.

Author contributions

Study conception and design: J. Vera and M.C. Ruiz-Sánchez. Data curation: J.Vera , W. Conejero and A.B. Mira-García. Writing—original draft preparation: J. Vera M.C. Ruiz-Sánchez, A.B. Mira-García, and M. R. Conesa. All authors contributed commented on previous versions of the manuscript, read and approved the final manuscript. Supervision, project administration and funding acquisition: M.C. Ruiz-Sánchez

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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ARTICLE II:

Leaf water relations in lime trees grown under shade netting and open-air

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Objectives

The aim of this study was to assess the diurnal and seasonal variations of several plant water status indicators in lime trees growing under two cropping systems: open-field and shade netting.

Materials and Methods

The experiment was carried out during the 2018-2019 growing season at the experimental field station of CEBAS-CSIC in Murcia, Spain. The plant material comprised three-year old lime trees (*Citrus latifolia* Tan., cv. Bearss) grafted on *C. macrophylla* L. rootstock. Trees were planted in raised rows, spaced at 6 m x 5 m. The irrigation system consisted of a double drip lateral line, with four pressure-compensated emitters, of 4 L h^{-1} per tree. The trees were irrigated to fulfil plant water requirements throughout the experiment using an automated irrigation based on threshold values of volumetric soil water content (θ_v) measured with TDR probes. The threshold values of θ_v were fixed to 10 % of management allowed depletion and field capacity to trigger and stop irrigation, respectively. The trees were subjected to two cropping systems: open-field (as the most common agricultural practice) and shade netting (trees grown inside a shade net house).

Meteorological variables such as precipitation, air temperature (T_a), relative humidity (RH), wind speed and solar radiation, were recorded in open-field and shaded conditions by two automatic weather stations. Photosynthetically active radiation (PAR) was measured by a portable photosynthesis system. On a typical summer and winter day, discrete measurements of stem water potential (Ψ_{stem}) by a pressure chamber, and leaf gas exchange (stomatal conductance (g_s) and net photosynthesis (P_n)) with a portable photosynthesis system, were made at 1-2 h intervals. Canopy temperature (T_c) was continuously monitored these days with infrared radiometer sensors. Canopy to air temperature difference (T_c-T_a) and the canopy thermal index (CTI) were calculated. Plant water status was assessed in four trees per cropping system, each considered as a replication.

Results and Discussion

Meteorological conditions were more demanding in summer (mean T_a of 26 ◦C) than in winter (mean T_a of 14 ◦C) season. In summer, T_a inside shade net house was

slightly higher than in open-field, favouring a reduction of RH values. In winter, T_a and RH were very similar in the two cropping systems studied. Regardless of the season, PAR under netting was lower than in open-field, due to light scattering effect of the net.

Plant water status indicators showed diurnal and seasonal changes. In both cropping systems, Ψ_{stem} followed a circadian pattern closely related with vapor pressure deficit (VPD) changes. In summer, the diurnal values of Ψ_{stem} fluctuated more than in winter. Leaf gas exchange parameters diurnal course showed high values early in the morning and minimum at midday. In summer, accumulated daily g_s and P_n were 54 and 37 %, respectively, higher in shade netting than in open-field conditions. The low PAR under netting, resulted in high stomatal conductance, which could improve CO_2 uptake, promoting an increase of photosynthetic capacity of shaded trees. In winter, leaf gas exchange was similar in both cropping systems. T_c showed higher values in summer, varying from 20 to 40 ◦C, than in winter, varying from 3 to 20 ◦C. In both seasons, trees grown in shaded conditions showed at midday slightly higher T_c values respect to open-field trees. This fact could be due to the high T_a and low RH values measured inside the shade net house, promoting a canopy warming. Shaded lime trees, however, showed at midday lower values of T_c-T_a than in open-field conditions, especially in summer. This reduction in T_c-T_a observed under netting led to a high degree of leaf cooling in this cropping system. In the two seasons, CTI was lower in shaded than in open-field during most of the day. CTI and Ψ_{stem} showed a close relationship.

Conclusions

Plant water status indicators measurements were affected by diurnal and seasonal changes. The studied indicators showed different diurnal circadian rhythms, with maximum g_s, P_n and T_c, and minimum Ψ_{stem} values recorded at midday. These plant indicators were also affected by seasonal variations showing low values in winter and high in summer. Cropping systems mainly affected leaf gas exchange of lime trees, improving the g_s and P_n values of shaded trees. The monitoring of T_c, T_c-T_a and CTI could serve as a good alternative to other traditional plant water status indicators.

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Article

Leaf Water Relations in Lime Trees Grown under Shade Netting and Open-Air

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Abstract: Physiological plant water status indicators are useful for managing precision irrigation in regions with limited water resources. The aim of this work was to evaluate the effect of shade netting on the diurnal and seasonal variations of several plant water status indicators in young lime trees (*Citrus latifolia* Tan., cv. Bearss), grown at the CEBAS-CSIC experimental station in Murcia, Spain. Stem water potential (Ψ_{stem}), leaf gas exchange (net photosynthesis (P_n) and stomatal conductance (g_s)), and canopy temperature (T_c) were measured on representative days of winter and summer. The Ψ_{stem} daily pattern was quite similar in both seasons under both conditions. However, the circadian rhythm of leaf gas exchange was affected by shade conditions, especially in summer, when shaded leaves showed maximum g_s values for a longer time, allowing higher net photosynthesis (37%). Canopy temperature behaved similarly in both conditions, nevertheless, lower values were recorded in open-air than in shaded trees in the two seasons. The canopy-to-air temperature difference (T_c-T_a), however, was lower in shaded trees during the daylight hours, indicating the higher degree of leaf cooling that was facilitated by high g_s values. The possibility of continuously recording T_c makes it (or the proposed canopy thermal index, CTI) a promising index for precise irrigation scheduling. Shade netting was seen to favour gas exchange, suggesting that it may be considered alternative to open-air for use in semi-arid areas threatened by climate change.

Keywords: canopy temperature; drip irrigation; leaf water relations; lime trees; net house; thermal canopy index

1. Introduction

Citrus is one of the most important crops in the world, with an annual production of 124 Mt, increasing by 10 % over the last five years. Spain is the most important citrus fruit producer in Europe and the largest citrus exporter in the world (4.1 Mt) [1]. Citrus production is concentrated in the Comunidad Valenciana (3.1 Mt), Andalucía (2.2 Mt) and Region de Murcia (0.8 Mt) [2]. In Murcia, lemon has traditionally been the main citrus species (representing 64 % of the province's citrus production) [3], but in recent years others species such as lime have contributed to diversifying production, as growers seek a higher economic return than that offered by traditional local citrus varieties. Indeed, lime production in the region has increased by 25% in the last three years [4] and has become a good potential alternative for growers.

In forthcoming years, climate change is set to increase temperatures and hence evaporation [5], reducing water availability to an even greater extent, having a detrimental effect on fruit tree production worldwide. Under this scenario, especially in areas with limited water resources such as those in Mediterranean environments, the use of physiological water status indicators for precision irrigation management is considered a suitable way of adjusting the provided water to cover the needs of plants, thus saving significant amounts of water. Plant-based indicators rely on using the

plant as a biosensor that integrates the soil and atmosphere water status, as well as measuring the physiological response to the water available in the soil. The most commonly used are the conventional, non-automated methods for measuring leaf or stem water potential, stomatal conductance or photosynthesis, and automated methods for measuring sap flow, canopy temperature, trunk diameter, or leaf turgor pressure [6]. Canopy temperature is one of the plant water status indicators that has recently become popular due to the development of infrared thermometry [6,7]. In the last few years, the use of canopy temperature for irrigation scheduling has been tested in several fruit tree crops, including cherry [8,9] avocado [10], almond [11], nectarine [12], peach [13] and also citrus trees such as lemon [14,15]. However, less information is available concerning lime.

Shading, which aims to protect crops against abiotic stresses such as high air temperature or excessive solar radiation, is regarded as a good cropping technique to cope with climatic change. This is likely due to the net increases light scattering, thus reducing radiation and creating an optimal microclimate for crop development [16]. As several studies have pointed out [17,18], shaded conditions affect agrometeorological variables, such as relative humidity, wind velocity and canopy and soil temperatures. Additionally, changes in the microclimate can modify physiological water status indicators. Many authors have pointed to the increased photosynthetic activity that occurs under reduced radiation, although it must be remembered that net-type affects this response [19–21]. The increase in CO_2 uptake [22] and chlorophyll synthesis [20] induced by shading has been seen to increase tree growth and fruit yield, as reported for several citrus and other woody tree species [23–25]. Moreover, shade netting improves the quality of the fruit by boosting protection against biotic stress (diseases, insect pest) [26]. The ability of shade netting to improve water use efficiency, among other physiological traits, makes it a sound agricultural practice in areas with limited water sources.

For these reasons, the aim of this study was to evaluate the diurnal and seasonal variations of several plant water status indicators in lime trees, making discrete measurements of stem water potential (Ψ_{stem}) and leaf gas exchange (stomatal conductance (g_s) and net photosynthesis (P_n)), and continuous measurements of canopy temperature (T_c) in a Mediterranean environment of south-east Spain under two growing conditions: shade netting and open-air.

2. Results and Discussion

2.1. Meteorological Conditions

As is typical of Mediterranean climates, the meteorological conditions in the study area were more demanding in summer than in winter, with mean air temperature (T_a) of 26 °C in summer (mean maximum and minimum of 32 and 21 °C, respectively) and 14 °C in winter (mean maximum and minimum of 16 and 9 °C, respectively). Air temperature and relative humidity (RH) were slightly different in the two cropping conditions in both seasons (Figure 1). In summer (August), the mean open-air T_a was lower (27.1 °C) and mean RH higher (65.5%) than in the shaded conditions (27.6 °C and 64.1%, respectively). Although only slight differences were noted in the mean values, the greatest differences were found at midday, coinciding with maximum T_a and minimum RH values (Figure 1A,B). Less pronounced differences between cropping conditions were noted during winter (February) with mean T_a = 12.1 and 12.0 °C, and mean RH = 61.5 and 62.8%, for open-air and shaded conditions, respectively. In this season, shaded conditions induced lower T_a than the open-air conditions during the night, but slightly higher values during daylight hours (Figure 1C,D).

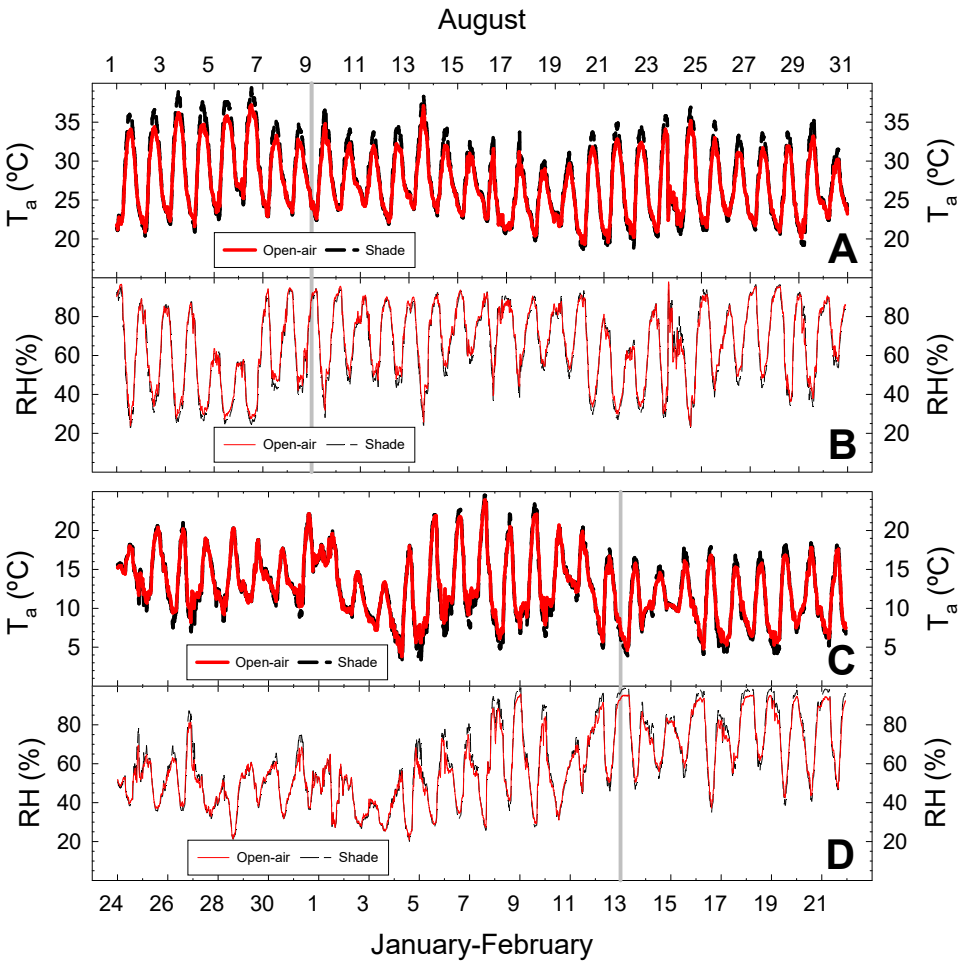

Figure 1. Diurnal pattern of air temperature (T_a) and relative humidity (RH) during representative months of summer (August 2018; **A,B**) and winter (January-February 2019; **C,D**) in open-air (solid line) and under shade netting (dashed line). Vertical grey lines indicate days of measurements (August 9th 2018 and February 13th 2019).

On the selected measurement days (August 9th 2018 and February 13th 2019), T_a varied between 22.6 ˚C and 36.8 ˚C in summer and from 4.6 to 15.8 ˚C in winter, in open-air conditions (Figure 2E,F). The vapour pressure deficit (VPD) showed a similar pattern, with maximal values in summer (up to 4 kPa) and minimal values in winter (below 1 kPa) (Figure 2C,D). However, photosynthetically active radiation (PAR) was quite similar in both seasons, with maximum values (≈ 1900 μmol m^{-2} s^{-1}), but with lower daylight hours in winter (Figure 2A,B).

Regarding the cropping conditions, the greatest differences in the agrometeorological variables were noted at midday, with higher T_a and VPD values in the shaded than in open-air conditions (Figure 2). In the summer, the maximum T_a was 2.0 ˚C lower in the open-air than under shade netting (Figure 2E), and 1.0 ˚C lower in winter (Figure 2F). Similar results were reported by Kührt et al. [27] in apple trees and Alaphilippe et al. [26] in pear trees, who observed that the air temperature in net conditions during daylight hours was 0.7 ˚C higher. However, the contrary was described by Blakey et al. [24] who observed that the air temperature was 2–3 ˚C lower in the shaded conditions to cultivate mango trees, while Gimeno et al. [28] observed a reduction up to 6 ˚C under the net shading cover provided for lemon trees. Blakey et al. [24] and Gimeno et al. [28] observed a reduction in the air temperature of 2–3 ˚C and 6 ˚C in mango and lemon trees, respectively under shaded conditions. Although all netting is intended for protecting trees and fruits from sunburn, mechanical injury from hail and wind, or other biotic/abiotic stresses, the contrasting results could arise from the different characteristics of the nets (net colour, shading factor, among others) [17,18].

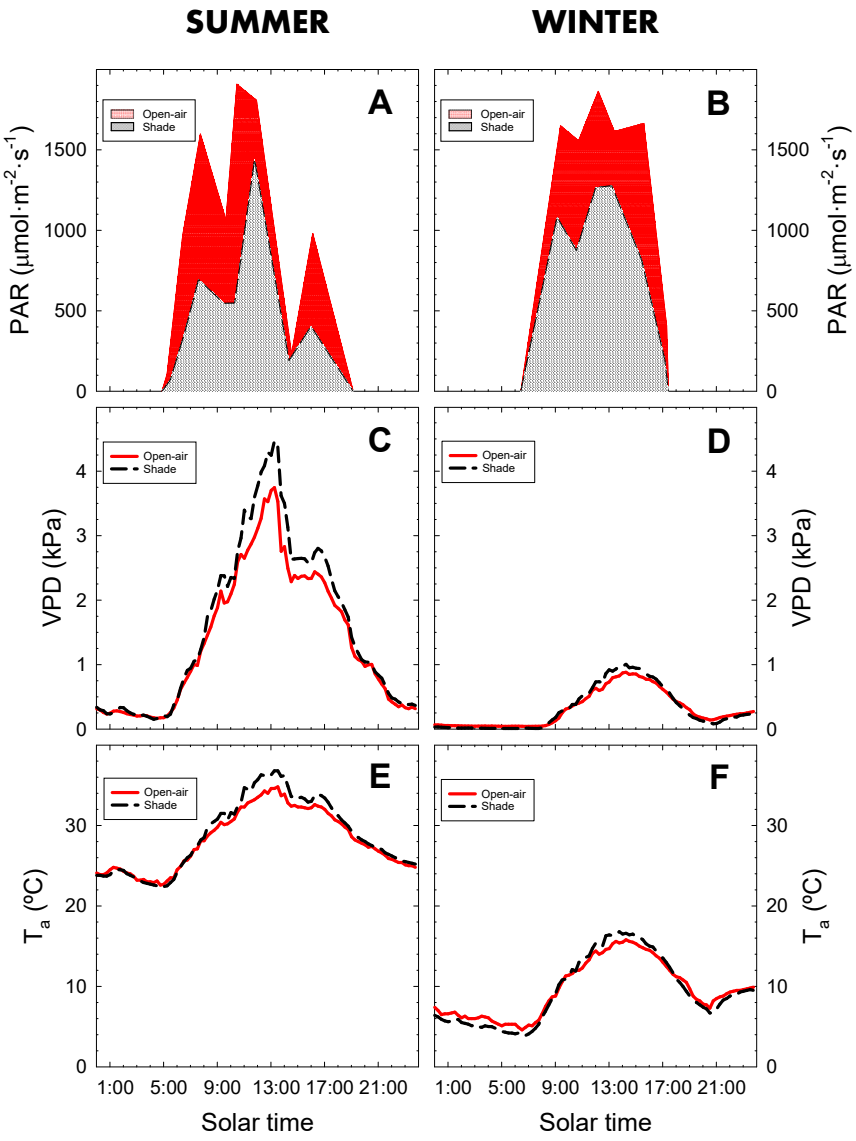

Figure 2. Diurnal course of incident photosynthetically active radiation (PAR), vapour pressure deficit (VPD) and air temperature (T_a) on representative days of summer (August 9th 2018; **A,C,E**) and winter (February 13th 2019, **B,D,F**) in lime trees grown in open-air (solid line) and shaded (dashed line) conditions. Lines are the 15-min average values.

As indicated above (Figure 1), RH values at midday were slightly lower in shade (mean 30%) than in open-air (33%) conditions. Similarly, Hunsche et al. [29] and Solomakhin and Blanke [30] observed a reduction (2–5%) in RH under netting. As a result of the higher T_a and lower RH in shade conditions, VPD values were higher than in the open-air conditions, especially in summer when the atmospheric conditions were more demanding (Figure 2C,D). This fact could be mediated by the semi-permeable characteristics of the net and the architecture of the net-house (see Materials and Methods) that caused less air renewal and, therefore, reduced the advective effect inside the net house.

During night-time hours, T_a values were similar in both cropping conditions in summer (Figure 1A,2E), although there were some differences in the winter, when T_a was lower (≈1.5 °C) in shaded than in open-air conditions (Figures 1C, 2F). A slight decrease in the air temperature (0.4 °C) during the night hours was noted under anti-hail black netting [27]. However, no differences were noted in VPD values between cropping conditions at this time in either season (Figure 2C,D).

PAR values were significantly lower inside the net house than in open-air conditions. The area under the PAR curves (Figure 2A,B) shows that net shading reduced the level of radiation by 53 %

and 30 % in summer and winter, respectively. García-Sánchez et al. [31] observed a substantial (≈50%) reduction in PAR values in shaded lemon trees, compared with the 39 % reduction observed in orange trees by Zhou et al. [21]. This reduction in the incident PAR in the shade would be due to the light scattering effect of the net, which disperses the light and only allows part of incident radiation through.

2.2. Stem Water Potential

During the experimental period, the trees were not affected by limiting soil water conditions, as the stem water potential (Ψ_{stem}) confirmed, with values > −0.9 MPa (Figure 3A,B), indicating that the plant did not suffer water stress [15,32,33]. As in most cultivated plants, Ψ_{stem} in both cropping conditions showed a circadian pattern characterized by higher values at the beginning of the day, after which the values gradually decreased as evaporative demand increased, reaching a minimum at around midday [15,19,34,35]. After midday, as a consequence of a decrease in the evaporative demand, the Ψ_{stem} gradually increased until the end of the day (Figure 3A,B).

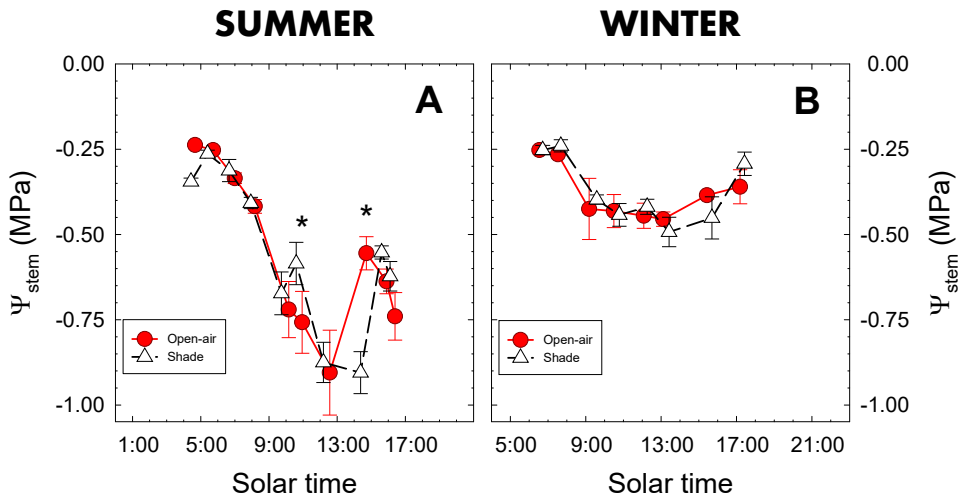

Figure 3. Diurnal course of stem water potential (Ψ_{stem}) on representative days of summer (August 9th 2018; **A**) and winter (February 13th 2019; **B**) in lime trees grown in open-air (closed symbols) and shaded (open symbols) conditions. Each point is the average of four replications. Vertical bars represent ± standard error (SE). Asterisks indicate statistically significant differences between cropping conditions at $p \leq 0.05$.

In summer Ψ_{stem} values fluctuated between −0.25 and −1.0 MPa, while in winter between −0.25 and −0.5 MPa. In winter, no statistically significant differences were observed between cropping conditions. However, in summer, significant differences were observed around 11:00 and 15:00 h solar time when shaded trees showed lower Ψ_{stem} values than open-air trees (Figure 3A,B). The more negative Ψ_{stem} values coincided with the higher VPD values (Figure 2C,D), likely due to the linking between Ψ_{stem} and evaporative demand of the atmosphere. In our experimental conditions, Ψ_{stem} was closely related to VPD, as demonstrated by the strong correlation between VPD and Ψ_{stem} (Figure 4). Higher determination coefficient values (R^2) were found when open-air and shade net conditions were considered separately but the covariance analysis indicated no differences between the regression coefficients. Similar relationships have been observed in studies made in lemon [15], peach [36], apricot [35], almond [37] and avocado [10], highlighting the role of the atmosphere as a driving force for the water transference processes, and emphasizing the suitability of Ψ_{stem} as a robust plant water status indicator [38–40].

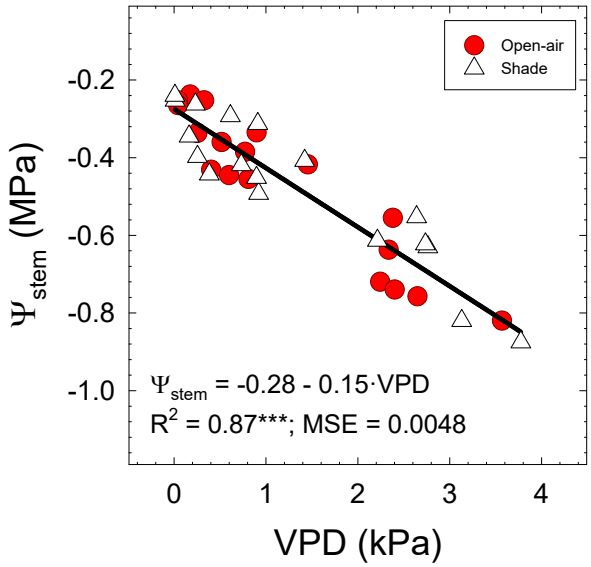

Figure 4. Relationship between daily stem water potential (Ψ_{stem}) and vapour pressure deficit (VPD) values in lime trees grown in open-air (closed symbols) and shaded (open symbols) conditions on the two measurement days. Each point is the average of four replications. *** $p \leq 0.001$. MSE = mean squared error.

2.3. Gas Exchange

The diurnal courses of leaf gas exchange (stomatal conductance (g_s) and net photosynthesis (P_n)) pointed to maximal stomatal opening during the early morning hours and minimum values at midday [41] in both conditions, as illustrated in Figure 5. In summer, g_s in open-air grown trees increased rapidly and then decreased rapidly, whereas shaded trees maintained significantly higher stomatal conductance values until midday (Figure 5A), resulting in a 54 % higher accumulated daily g_s compared with open-air trees. In the same way, an increase of stomatal conductance (more than 50 %) was observed in several apple tree cultivars grown under black shade net compared with those grown in open-air conditions [42].

A similar daily pattern was observed for net photosynthesis in summer, with significantly higher mean daily values (37 %) in shaded trees, although a delay in reaching maximum P_n values was noted with respect to open-air trees (Figure 5C). These higher leaf gas exchange levels under shade conditions could be due to the lime trees being exposed to lower incident PAR radiation (Figure 2A), and such non-saturated conditions would allow the stomata to remain open longer (Figure 5A). The higher stomatal opening would improve CO_2 uptake [22], increasing the photosynthetic capacity of shaded trees, as several studies have reported [16,20,43]. It should be mentioned that a cloudy event (around 15:00 solar time) caused an abrupt decrease in PAR (Figure 2A) and VPD (Figure 2C) values. This unexpected condition did not consistently affect the leaf gas exchange parameters (Figure 5A,C), which were following the circadian rhythm of stomatal cycling (Figure 5A) as has been similarly reported in citrus species [34,44–46].

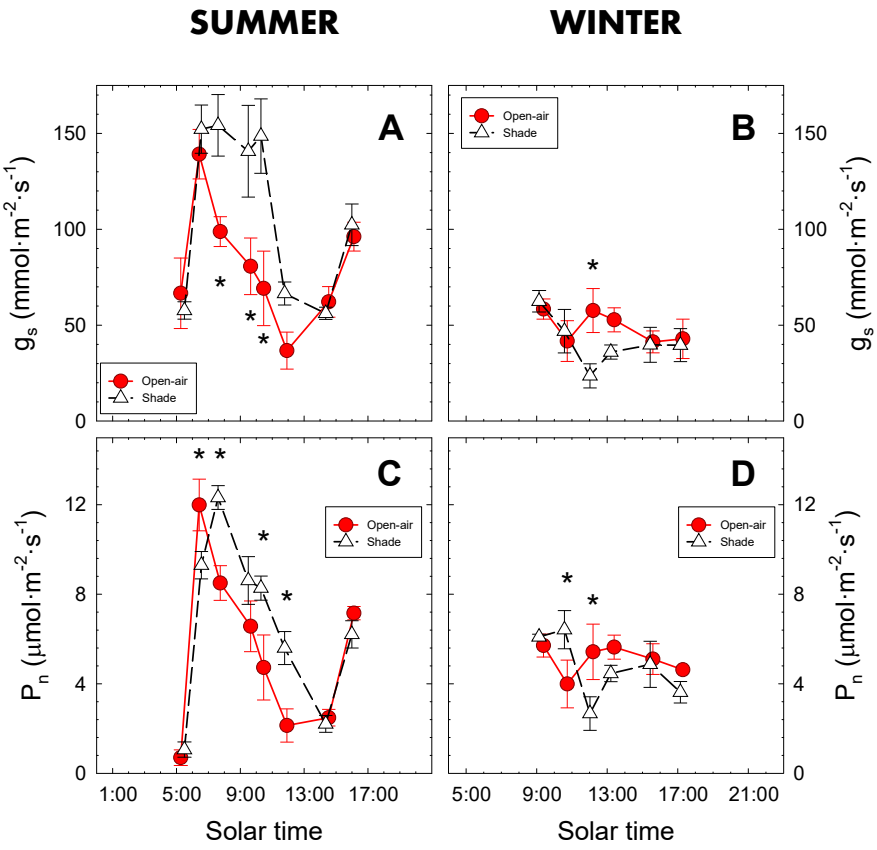

Figure 5. Diurnal course of stomatal conductance (g_s) and net photosynthesis (P_n) on representative days of summer (August 9th 2018; **A** and **C**) and winter (February 13th 2019; **B** and **D**) in lime trees grown in open-air (closed symbols) and shaded (open symbols) conditions. Each point is the average of four replications. Vertical bars represent ± standard error (SE). Asterisks indicate statistically significant differences between cropping conditions at $p \leq 0.05$.

In winter, the less demanding climatic conditions led to lower gas exchange values, which behaved similarly in both cropping conditions, with only slight significant differences before midday when trees cultivated in the open-air had slightly higher values than the trees grown in shaded conditions (Figure 5B,D).

The climatic conditions clearly influenced gas exchange [44], but may also have been affected by the feedback effect of the fruits on leaf photosynthesis [47], with higher P_n and g_s values recorded in August than in February (Figure 5). The presence of fruits at their maximum growth rates just before harvesting (at the end of August 2019) would have been responsible for this behaviour, as found in *Citrus* [15] and *Prunus sp.* [35,48].

The daily gas exchange dynamics in both cropping conditions showed maximum stomata opening and higher net photosynthesis values early in the morning, as seen in other studies [33], confirming that leaf gas exchange in lime trees should be measured at this time of the day for comparative purposes. Moreover, based on the higher correlations found with maximum daily g_s values [15], Ψ_{stem} should be measured at midday.

2.4. Canopy Temperature

The development of infrared thermometry techniques has made it possible to measure canopy temperature remotely, without any physical contact with the plant. The continuous canopy temperature (T_c) records for the lime trees showed values varying from 20 to 40 °C in summer, and from 3 to 20 °C in winter (Figure 6A,B). The minimum T_c values were recorded at the beginning and the end of the day, and maximum T_c values around midday. In both seasons, the canopy

temperature from dawn to dusk was very similar in both cropping conditions. However at midday, when the meteorological conditions were more demanding (Figure 2), plants grown in shade conditions had a higher T_c (2.70 and 2.65 °C, in summer and winter, respectively) than in those grown in open-air conditions (Figure 6A,B). Blackey et al. [24] also observed an increase of 10 % in maximum T_c in avocado as a result of shade netting. However, in orange trees [21], canopy temperature was similar in non-netted (35.4 °C) and shaded (35.3 °C) trees. The slight increase in the T_c of shaded lime trees (Figure 6A,B) could have been due to the high T_a and low RH values measured inside the net house at midday in both seasons (Figure 1). However, it should be noted that the microclimate of the leaf boundary layer was slightly different from the measurements of the climatic sensors (Figure 2C,D), with lower VPD_{leaf} values in shaded than in open-air conditions, as measured by the LICOR, LI-6400.

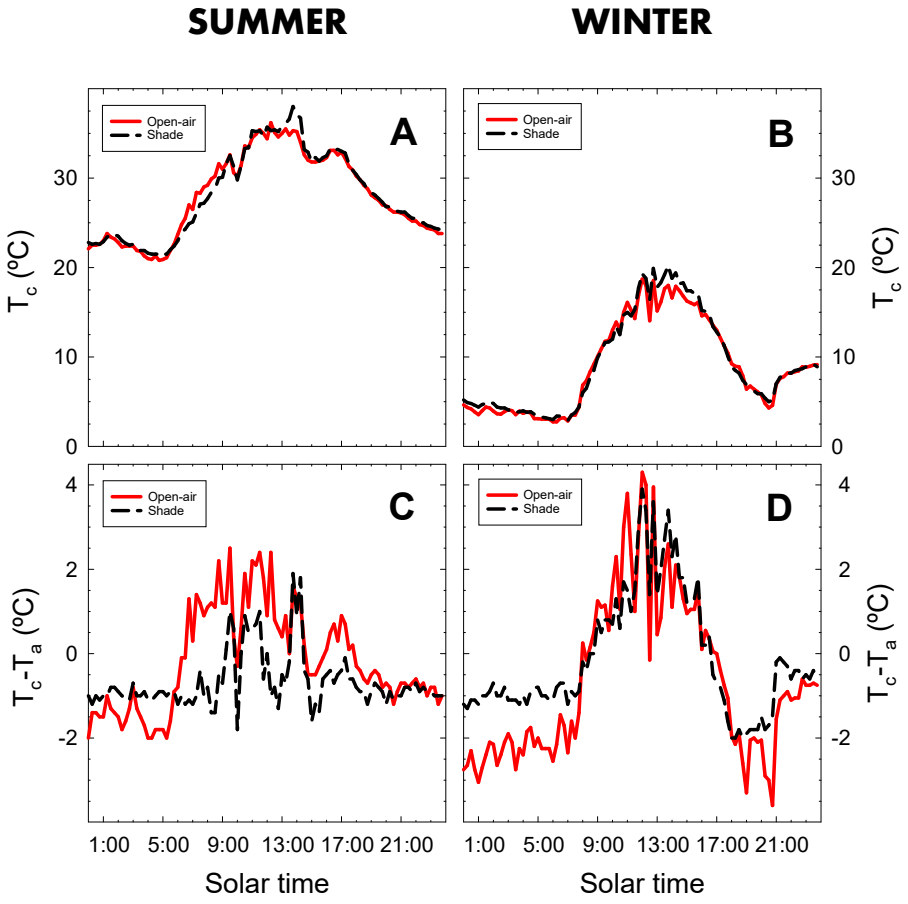

Figure 6. Diurnal course of canopy temperature (T_c) and canopy-to-air temperature difference (T_c-T_a) on representative days of summer (August 9th 2018; **A** and **C**) and winter (February 13th 2019; **B** and **D**) in lime trees grown in open-air (solid line) and shaded (dashed line) conditions. Lines are the 15-min average values of two replications.

Shaded lime trees, however, showed lower values for the canopy-to-air temperature difference (T_c–T_a) than the trees grown in open-air conditions, particularly in summer, when T_c–T_a was negative during the greatest part of daylight hours (Figure 6C). This reduction in T_c–T_a led to a higher degree of leaf cooling in shaded trees as a consequence of the increase in stomatal conductance in the shade net conditions (Figure 5A). In winter, T_c–T_a values were quite similar in the two cropping conditions during daylight hours, while lower values were noted in open-air than in shaded trees during the night (Figure 6D).

Canopy temperature is recognized as a sound plant water status indicator [6,7,49] and is affected by the prevailing environmental conditions. Rather than absolute T_c values, the difference

between the canopy and air temperature has been proposed as a sound indicator of plant water status. High T_c-T_a values indicating water stress and low values well-irrigated conditions [50,51]. The higher T_c-T_a values measured in lime trees grown in the open-air than in shaded trees (Figure 6C) would result from stomatal closure (Figure 5A).

The proposed canopy thermal index (CTI) based on the normalised T_c-T_a values (equation 1) under the two cropping conditions during one week of summer and winter is presented in Figure 7. The CTI pointed to lower values in shaded than in open-air grown trees during most of the day (Figure 7). When the respective measurement days are examined, a close relationship can be seen between CTI and plant water status - the higher the CTI the lower the Ψ_{stem} -, as observed in predawn on August 9th (Figure 3A) and during the early afternoon of February 13th (Figure 3B). Additionally, the higher CTI values would explain the higher T_c recorded in shaded plants in both seasons (Figure 6A,B).

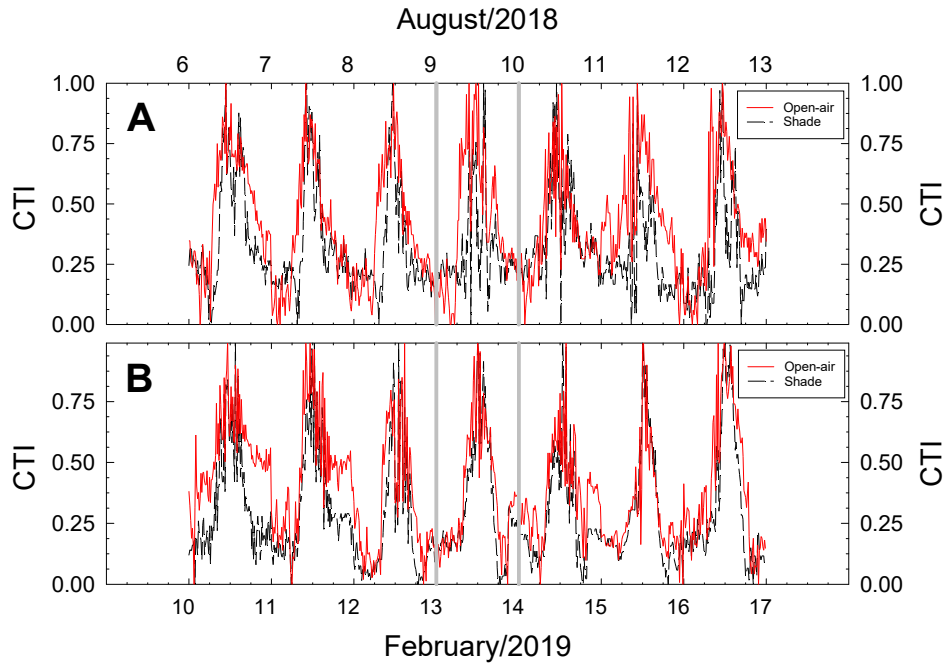

Figure 7. Canopy thermal index (CTI) for representative weeks of summer (6–13 August 2018; **A**) and winter (10–17 February 2019; **B**) in lime trees grown in open-air (solid line) and shaded (dashed line) conditions. Vertical grey lines indicated the measurement days (August 9th 2018 and February 13th 2019). Lines are the 15-min average values of two replications.

The integrated daily CTI values ($\sum_{24h}$CTI) were 7.24 and 10.00 for shaded and open-air trees, respectively, on the above mentioned day in summer, and 7.56 and 8.93 in winter. This behaviour was maintained when the weekly data in Figure 7 were analysed, statistically significant differences between cropping conditions being evident: an average $\sum_{24h}$CTI of 7.57 ± 0.44 and 10.02 ± 0.39 for shaded and open-air trees in August, and 7.04±0.36 and 9.49±0.75 in February, respectively.

Although more studies are required, the fact that low CTI values were associated to better plant water status (lower Ψ_{stem}) empowers the sensor-based of continuous T_c measurements as a sound water status indicator in lime trees, thus canopy temperature, in terms of CTI, is proposed as a tool for irrigation management in areas with scarce water resources.

3. Materials and Methods

3.1. Plant Material and Experimental Conditions

The experiment was carried out during the 2018–2019 growing season at the experimental field station of CEBAS-CSIC in Murcia, Spain (38° 06′ 31″ N, 1° 02′ 14″ W, 110 m a.s.l.). The plant material comprised three-year-old lime trees (*Citrus latifolia* Tan., cv. Bearss) grafted on *Citrus macrophylla L.* rootstock. The soil of the 0–0.5 m layer was stony, highly calcareous (45% calcium carbonate), with a clay loam texture and low organic matter content. The average bulk density was 1.43 g cm^{-3}. The soil water content at field capacity and permanent wilting point were 0.29 and 0.14 m^3 m^{-3}, respectively.

Trees were planted 5 m apart in raised rows (2 m wide and 0.4 m high), which were 6 m apart. The trees were irrigated with a double drip lateral line separated 1 m, with four pressure-compensated emitters, of 4 L h^{-1}, located 0.7 m from the tree trunk. The trees were irrigated to fulfil plant water requirements throughout the experiment. The irrigation scheduling was automated with soil management that allowed depletion (MAD) of 10% [52]. The soil water content was monitored at a depth of 0.8 m by means of multi-depth capacitance probes (EnviroPro®, Entelechy Pty. Ltd., Golden Grove, Australia) and the measurements served as feedback for irrigation control by acting on electrovalves that formed part of a telemetry system. The values were read every 5 min and the averages were recorded every 15 min. The radio-transmission units sent data to a gateway connected to addVANTAGE web server software (ADCON Telemetry, Austria) for data processing and visualisation. Eight soil probes were calibrated and installed 0.1 m from the selected emitter of representative trees (four for each cropping condition).

3.2. Cropping Conditions

The trees were subjected to two cropping conditions: open-air (Figure 8A) and shade netting (Figure 8B), each consisting of 60 trees. Both cases received the same amount of irrigation water. The shade netting formed a net house 4.5 m high, with a zig-zag roof (Figure 8B). The HDPE white agricultural net used had a 76 % light transmission and 6 × 9 strands cm^{-2} (CYLNET).

Four representative mid-row trees were selected to monitor each cropping condition, the rest serving as border trees.

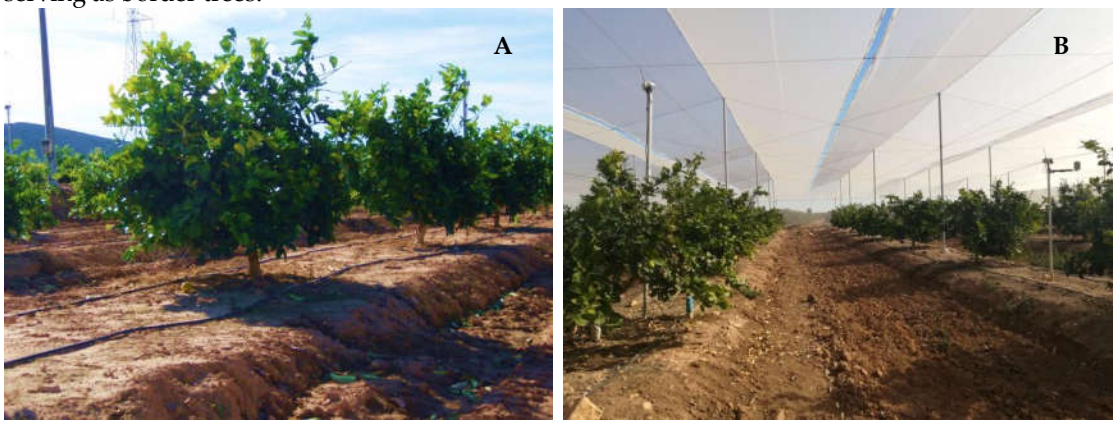

Figure 8. General view of lime trees (*Citrus latifolia* Tan., cv. Bearss) grown in open-air (**A**) and shade net house (**B**) conditions.

3.3. Measurements

Precipitation, air temperature, relative humidity, wind speed, and solar radiation were recorded in real-time by an automatic weather station located at the experimental site (http://www.cebas.csic.es/general_spain/est_meteo.html). Additionally, a combined air temperature and relative humidity sensor was installed at a height of 2 m in the middle of the shading net house. The vapour pressure deficit (VPD) for open-air and shade net were calculated hourly. The

photosynthetically active radiation (PAR) was obtained from the measurements made by the quantum-sensor mounted in the chamber of the photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA).

A typical summer (9th August 2018) and winter (13nd February 2019) day, representative of high and low atmospheric water demand conditions, respectively, served to evaluate plant water status. Discrete measurements of stem water potential (Ψ_{stem}) and leaf gas exchange were made at 1 to 2 h intervals. Continuous records of canopy temperature (T_c) were recorded.

The Ψ_{stem} was measured with a pressure chamber (Soil Moisture Equipment Corp., Model 3000, Santa Barbara, CA, USA) in mature leaves located near the trunk on the north face of the trees. One leaf per tree (four trees per cropping condition) was covered with aluminium foil at least 2 h before excision, following the recommendations described in [53].

Fully expanded (3 to 5 months old), sun-acclimated leaves (one leaf per tree and four trees per cropping condition, from the exterior canopy were used for gas exchange measurements: leaf net photosynthetic rate (P_n), and stomatal conductance (gs) were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a broad-leaf chamber (6 cm^2). Measurements were taken at near-constant ambient CO_2 concentration (≈ 400 μmol mol^{-1}) and the airflow rate inside the chamber was about 350 μmol s^{-1}.

Canopy temperature (T_c) was monitored using infrared radiometer sensors (Model SI-431 series, Apogee Instruments, Inc., Logan, UT, USA). The sensors were mounted on galvanized metal poles and installed 0.5 m above the tree canopy on two representative trees for each cropping condition. The half field of view (FOV) of these sensors was 14°, covering an area of 488 cm^2 of the tree canopy. The IRT sensors were calibrated by the manufacturer. They were connected to the wireless sensor network, reading the values every 5 min and recording average values every 15 min.

The average values of T_c were computed and T_c–T_a values were calculated for open-air and shade conditions. The following index is proposed, based on 15-min lapsed T_c–T_a values. The canopy thermal index (CTI) was formulated as:

$$CTI = \frac{(T_c - T_a) - (T_c - T_a)_{min}}{(T_c - T_a)_{max} - (T_c - T_a)_{min}} \quad (1)$$

where, $(T_c–T_a)_{min}$: minimum T_c–T_a daily value and $(T_c–T_a)_{max}$: maximum daily T_c–T_a value. The index varies between 0 and 1.

3.4. Statistical Analysis

Data were analysed using IBM SPSS Statistic software (version 25, SPSS Inc., Chicago, IL, USA 2002). Analysis of variance was performed and the mean values were compared by LSD0.05 test. Relationships between parameters were fitted to linear regressions.

4. Conclusions

The behaviour of plant water status indicators in lime trees and agro-meteorological variables were affected by both diurnal and seasonal changes and cropping conditions. The studied indicators identified different circadian rhythms, with maximum gas exchange and canopy temperature, and minimum stem water potential values, recorded at midday. Moreover, these plant indicators affected by seasonal variations had lower values in winter when the atmospheric demand conditions were also lower. The cropping conditions mainly affected the gas exchange of young lime tree leaves, the stomata of plants cultivated in the shaded conditions closing later and reaching maximum values after the plants cultivated in open-air. This would favour plant cooling, as indicated by the more negative values of the canopy-to-air temperature difference in summer. Then, shade netting is an interesting cropping technique and increasingly recognised as an alternative to open-air in semi-arid areas threatened by climate change, as the resulting increase in leaf gas exchange would favour carbon assimilation.

Due to the circadian rhythms of the plant water status indicators in leaves, we recommend that the stem water potential be determined at midday, coinciding with the maximum evaporative

demand of the atmosphere and that gas exchange is measured early in the morning before the stomata of the lime leaves close.

The continuous measurement of canopy temperature using robust wireless infrared radiometers could serve as a good alternative to other classical physiological measurements for measuring plant water status. Although more research is needed on the use of ground-based thermal imagery to detect water stress threshold values in lime trees, the proposed canopy thermal index (CTI), virtually independent of the season of the year, could be used for tuning irrigation scheduling in areas where the scarcity of water resources demands precise irrigation.

Author Contributions: Conceptualization and investigation, A. B. M.-G., W.C., J.V. and M.C.R.-S.; data curation, A. B. M.-G. and W.C.; writing—original draft preparation, A. B. M.-G., and M.C.R.-S.; writing—review and editing, A. B. M.-G., W.C., J.V. and M.C.R.-S.; supervision, project administration and funding acquisition M.C.R.-S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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ARTICLE III:

Water status and thermal response of lime trees to irrigation and shade screen

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Objectives

The main purposes of this study was: (i) to assess plant and soil water status and thermal response of lime trees to shading and soil water deficits conditions and (ii) to determine threshold thermal-based values for irrigation management.

Materials and Methods

The experiment was conducted during summer-autumn of 2021 at the experimental field station of CEBAS-CSIC in Murcia, Spain. The experimental plot of 0.8 ha was cultivated with Bearss lime trees (*Citrus latifolia* Tan.) grafted onto *Citrus macrophylla* L. rootstock. The trees were planted on 2016, on ridges at 6 m × 5 m tree spacing. They were irrigated with a double drip lateral line, with four pressure-compensated emitters, of 4 L h^{-1} per tree. Irrigation was automatically managed based on threshold values of volumetric soil water content (θ_v) measured with TDR sensors. The threshold values of θ_v were fixed to 30 % management allowed depletion and field capacity to trigger and stop irrigation, respectively. The trees were grown in two cropping systems: open-field and shade netting. Each cropping system was subjected to control (well irrigated) and stress (non-irrigated from 7th June to 24th September 2021) treatments. Environmental data in open-field and shaded conditions were real-time monitored by two automatic weather stations. Along the experimental period, plant water status was assessed by discrete measurements of stem water potential (Ψ_{stem}), by a pressure chamber, and net photosynthesis (P_n) and stomatal conductance (g_s), with a portable photosynthesis system. Additionally, canopy temperature (T_c) was continuously monitored with infrared radiometer sensors. Subsequently, canopy to air temperature difference (T_c-T_a), crop water stress index (CWSI), canopy thermal index (CTI) and daily integral thermal index (ΣT_c-T_a) were calculated. In addition, the relationship between Ψ_{stem} and T_c-T_a data in the two cropping systems studied was also assessed from an irrigation management point of view. Plant and soil water status were measured in four trees per irrigation treatment-cropping system, each considered as a replication.

Results and Discussion

Control trees of both cropping systems showed Ψ_{stem} values ranging from -0.59 to -1.19 MPa pointed to an optimal water status, as revealed the high θ_v values in the active root zone. Ψ_{stem} gradually fell in stressed lime trees as θ_v values decreased. In the two cropping systems studied, the soil drying curve showed a breaking point, indicating the

beginning of severe water stress in lime trees. This breaking point occurred earlier in open-field than in shaded conditions. When the soil water stress was moderate, lower values of Ψ_{stem}, θ_v, g_s and P_n, were observed in open-field than in shaded conditions, indicating that shade netting conditions allowed better soil-plant water status. When soil water stress was severe, these water status indicators showed similar and low values in both cropping systems. When the water restrictions ended, Ψ_{stem}, θ_v, g_s and P_n gradually increased to reach control values. T_c values were higher in stress than in control trees in the two cropping systems studied, since in stress conditions a stomatal closing was favoured, promoting a canopy warming. T_c-T_a values were the lowest in shaded conditions, indicative of good coupling plant-ambient in this cropping system. Higher values of CWSI, CTI and ΣT_c-T_a were observed in stressed trees than in control ones of both cropping systems indicating a worse plant water status.

The midday T_c-T_a threshold values proposed for triggering drip irrigation in lime trees grown under semi-arid conditions were 2.4 or 0.8 ◦C for open-field and shaded conditions, respectively, when irrigation water availability allowed to fulfil plant water requirements. Under deficit irrigation conditions, the proposed values were 2.9 or 1.3 ◦C, for moderate water deficits (Ψ_{stem} = -1.5 MPa) and 3.4 or 1.8 ◦C, for severe water deficits (Ψ_{stem} = -2 MPa), for open-field and shaded lime trees, respectively.

Conclusions

The response of lime trees to soil water deficit was different in the two cropping systems. Additionally, the ability to detect water stress also differed for the soil and plant water status indicators studied. Even though drawbacks, Ψ_{stem} was a reliable tool for assessing lime tree water status and along with the breaking point of θ_v was useful for irrigation monitoring. T_c-T_a dynamics reflected the “heartbeat” of lime trees. Soil water content sensors anticipated water stress detection than thermal-based ones. The high Ψ_{stem} g_s, P_n, and low T_c-T_a values observed under netting, revealed that shading allowed a better soil-plant water status. The cropping system must be considered to propose T_c-T_a threshold values for lime irrigation management.

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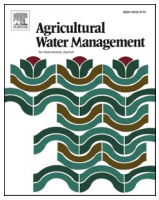

Water status and thermal response of lime trees to irrigation and shade screen

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ABSTRACT

Most lime trees are cultivated under open-field conditions. However, in the Mediterranean area some growers use shade screen to protect their orchards against abiotic and biotic stresses, inducing changes in the plant's surrounding microclimate that could affect its water status. This field experiment focuses on the effect of shade screen (high density polyethylene white net with 76% light transmission) and irrigation on plant and soil water status of Bearss lime trees. Two irrigation treatments were applied during the summer-autumn to open-field and shaded lime trees: irrigated (control) and non-irrigated (stress). Volumetric soil water content (θ_v), air temperature (T_a) and canopy temperature (T_c) were real-time monitored and several derived thermal indices were calculated. Stem water potential (Ψ_{stem}) and leaf gas exchange: net photosynthesis (P_n) and stomatal conductance (g_s) were measured on representative days. Withholding irrigation caused a gradual decline in soil and plant water status. θ_v decreased from the outset in the non-irrigated treatments, showing breaking point connecting the rapid and the slower soil drying processes. Significant differences in Ψ_{stem}, and g_s values were observed between controls and stressed trees 15 days after stress initiation. During early stages of water stress, lime trees cultivated in open-field showed lower values of soil and plant water status than those grown in shaded conditions. However, at the end of stress period, similar values were noted for both cropping systems, pointing to severe water deficit. T_c and T_c-T_a values reflected the current lime tree water status. The soil water content sensors anticipated the plant water stress situation respect to thermal ones. The higher θ_v, Ψ_{stem}, g_s, P_n and T_c values registered in shaded lime trees revealed that shading allowed better soil-plant water status and a 12-day window of opportunity to respond to the imposed soil water deficits. Threshold thermal-based values are proposed for precise irrigation management of lime trees in open-field and shaded conditions.

1. Introduction

Lime tree are gaining in importance as an agricultural crop. Although native to South-Central China, Brazil is nowadays the main lime producer, followed by Mexico, Iran, Egypt and Colombia (CIRAD, 2021). However, recently its cultivation has extended to the Mediterranean area, since it is well adapted to semiarid conditions. Due to climatic change (IPCC, 2021), the warmer climate may make this area even more beneficial for lime production, although the shortage of water means adopting more efficient water management and cultivation practices that promote water saving. Monitoring the parameters of the soil-plant-atmosphere system is decisive for evaluating the plant responses to irrigation regimes and crop cropping system, allowing the provided water to be adjusted to the plant's water requirements (Mira-García et al., 2020).

Soil water status was assessed by soil water content (θ_v) measurements, traditionally by gravimetric methods, which are destructive, time-consuming and not repeatable. Nowadays, other indirect methods based on the physical and chemical soil properties like time domain reflectometry (TDR), capacitance and impedance are gaining importance (Hardie, 2020; Vera et al., 2021). The plant itself has long been recognized as the best indicator of its water status, which can be assessed by direct measurements of leaf (Ψ_{leaf}) or stem water potentials (Ψ_{stem}), or indirectly by reference to leaf gas exchange parameters (stomatal conductance (g_s), net photosynthesis (P_n)), traditionally considered as good plant water status indicators for irrigation scheduling purposes (Cifre et al., 2005; García-Orellana et al., 2013; Hernandez-Santana et al., 2019; Mirás-Avalos et al., 2016). However, the draw-back of the manual measurements, is that they are laborious, destructive and time-consuming (Ballester et al., 2013b; García-Tejero et al., 2011).

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Furthermore, the progress made in telemetry have allowed real-time monitoring of other indirect plant-based water status indicators, such as trunk diameter shrinkage, sap flow or leaf turgor (Conejero et al., 2011; Fernández, 2017; Rodriguez-Dominguez et al., 2019). Also, canopy temperature (T_c), long recognized as an indicator of plant water status (Costa et al., 2013; Gates, 1964; Jones, 2004), can be *in-situ* or remotely measured by infra-red thermometry or thermal imaging, using infra-red radiometer sensors or thermal cameras. Thus, the measurement of T_c is a reliable, rapid and non-destructive technique for monitoring plant water status (Blaya-Ros et al., 2020; Leinonen et al., 2006; Romero-Trigueros et al., 2019). It is well known that T_c is influenced by atmospheric conditions such as vapour pressure deficit and radiation level, among others (Zhang et al., 2017). To attenuate the effect of these environmental variables on the absolute values of T_c different thermal indices have been proposed: the difference between canopy and air temperature (T_c-T_a), and the crop water stress index (CWSI) are the most performing (Idso et al., 1981; Jackson et al., 1981). Also, regression models have been suggested to predict leaf water potential based on T_c or CWSI values in *Prunus* (Tormann, 1986; Wang and Gartung, 2010), and *Citrus* (Cohen et al., 2005).

In addition, shade screen protect crops against abiotic stresses, such as adverse weather conditions, or biotic stresses such as pests and diseases (Mditshwa et al., 2019). This cropping system reduces the incoming radiation (García-Sánchez et al., 2015), affecting the microclimate surrounding the plants and hence their physiological behaviour (Manja and Aoun, 2019). In this sense, Incesu et al. (2016) observed an increase in the photosynthesis rate in 'Lane Late' oranges cultivated under black netting, while Jifon and Syvertsen (2003) observed lower T_c values in 'Red Ruby' grapefruit trees under aluminized screens. Whereas, shaded avocado trees showed increased T_c with respect to trees in open-field conditions (Blakey et al., 2016).

This study is aimed to assess plant/soil water status and thermal response of young lime trees to shading and soil water deficits. For this purpose, the following objectives were proposed: (i) to evaluate the ability of discrete (Ψ_{stem}, g_s and P_n) and continuous plant (T_c) and soil (θ_v) water status indicators to detect water stress, (ii) to identify the best performing thermal-based indices, and (iii) to determine threshold thermal-based values for irrigation management in semi-arid conditions. To do this, it was necessary to measure plant/soil water status and thermal-based indices under well-watered and water deficit conditions in shaded and non-shaded trees. We tested the hypothesis that microclimate caused by shade screen could affect these parameters in shaded *vs.* non-shaded lime trees. This information will be relevant in a future to manage the irrigation in a higher efficient way.

2. Materials and methods

2.1. Experimental site and plant material

The experiment was conducted during spring-summer-autumn of 2021 from DOY (day of year) 152–304 at the CEBAS-CSIC experimental field station located in Santomera, Murcia, southeaster Spain (38°06′31″ N, 1°02′14″ W, 110 m above sea level). The experimental plot of 0.8 ha was cultivated with 'Bearss' lime trees (*Citrus latifolia* Tan.) grafted onto *Citrus macrophylla* L. rootstocks, planted on 2016, on ridges (2 m wide and 0.4 m high) at 6 m × 4 m tree spacing. The soil had a clay loam texture, average bulk density of 1.43 g cm^{-3}, calcium carbonate content of 45 %, and organic matter content of 1.4 %. The volumetric soil water content (θ_v) at field capacity (FC) and permanent wilting point (WP) were 0.33 and 0.14 m^3 m^{-3}, respectively, obtained from the textural data (Saxton et al., 1986).

Trees were drip-irrigated with a double line per tree row separated 1 m from each other, with four pressure-compensated emitters (4 L h^{-1}) per tree, all located 0.7 m from the tree trunk. Irrigation was automatically managed in the two cropping systems by monitoring θ_v in the active root zone (0.2–0.6 m soil profile), following the approach described below for the control treatment (sub-Section 2.2). Fertilization and weed and pest control were carried out following the local practices of *Citrus* farmers.

2.2. Cropping systems and irrigation treatments

In this experiment two cropping systems were studied: open-field and shade screen, each subjected to irrigated and non-irrigated treatments:

- **Open-field cropping**: lime trees grown under natural ambient conditions, representing the most common agricultural practice (Fig. 1A).
- **Shade-screen cropping**: lime trees grown in a 2400 m^2 shade screen house, 4.5 m high and with a zigzag roof (Fig. 1B). The high density polyethylene white net used had 76 % light transmission and 6 × 9 strands cm^{-2}. Shade screen was installed on April 2018.

- **Irrigated treatment as "control"**: trees were irrigated to fulfil their water requirements throughout the experiment. The irrigation protocol was automated, based on real-time θ_v threshold values that acted on solenoid valves by means of a telemetry system. Volumetric soil water content was monitored by TDR sensors (model 315 H, Acclima, Inc., USA) horizontally installed 0.1 m from the emitter and closer to the tree trunk, on representative lime trees at depths of 0.2, 0.4, 0.6 and 0.8 m. The values were read every 5 min and the averages were recorded every 15 min. Radio-transmission units sent data to a gateway connected to an addVANTAGE cloud-based web server platform (ADCON Telemetry, Klosterneuburg, Austria) for data processing and visualisation. Threshold θ_v values were based on the management allowed depletion (MAD) concept (Allen et al., 1998; Merriam, 1966), as a percentage (α) of the total available soil water content (AWC) that can be depleted (MAD = α AWC), where AWC = FC – WP. Irrigation was automatically activated when mean θ_v values at 0.2–0.6 m soil depth (active root zone) reached 30 % of AWC, and stopped at FC.

- **Non-irrigated treatment as "stress"**: irrigation was withheld from 7th June to 24th September 2021 (DOY 158–267), coinciding with stage II of lime fruit growth. Before and after these dates, irrigation doses were the same as for control treatment.

Each cropping system consisted of a sub-plot of 60 lime trees. The irrigation treatments were distributed in shaded and open-field conditions in a completely randomised design, with four replications, each replication consisting of one row of six trees, with a total of 24 trees per irrigation treatment. One tree from each replication served for measurements and the rest of the plot were considered as borders.

2.3. Measurements

Environmental data, in both open-field and shaded conditions, were monitored by two automatic weather stations located in the both subplots. Values of air temperature (T_a), relative humidity (RH), wind speed, solar radiation and precipitation were read every 5 min and the averages were recorded every 15 min and transmitted to the cloud-based web server platform (sub-Section 2.2). The photosynthetically active radiation (PAR) was measured by the quantum-sensor mounted in the chamber of the portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE, USA). Also monitored were hourly crop reference evapotranspiration (ET_0), following Penman-Monteith' s equation and vapour pressure deficit (VPD) (Allen et al., 1998). Daily average VPD for each cropping condition was calculated from daily maximum and minimum T_a and RH data.

Volumetric soil water content (θ_v) was monitored through the 0.2–0.8 m soil profile, every 0.2 m, using Acclima-TDR 315H sensors,

Fig. 1. General view of lime trees (*Citrus latifolia* Tan. 'Bearss') grown in (A): open-field, and (B): under a shade screen.

horizontally installed 0.1 m away the emitter on representative trees (three out of four in each irrigation treatment and cropping system, n = 3). Values for θ_v were obtained as indicated above (sub-Section 2.2); a detailed description of the operation of these dielectric sensors can be found in Vera et al. (2021).

Stem water potential (Ψ_{stem}) was determined around midday (12:00 solar time) every ≈ 15 days on fully expanded leaves (one leaf per tree and replicate for each treatment and cropping system, n = 4), using a pressure chamber (model 3000, Soil Moisture Equipment Corp., California, USA) and following the recommendations of Hsiao (1990). Leaves, which had been bagged with foil-covered aluminium envelopes at least 2 h before the measurements were taken (McCutchan and Shackel, 1992), came from the north-facing side of the trees.

Leaf gas exchange, i.e. stomatal conductance (g_s) and net photosynthetic rate (P_n), were measured on the same days and in the same trees as Ψ_{stem}, at around 08:00 solar time, with a portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE, USA) equipped with a broad-leaf chamber (6 cm^2) and CO_2 injector. Measurements were taken at a constant light intensity (≈1200 μmol m^{-2} s^{-1}) provided by the red and blue LED source mounted on the leaf chamber. The CO_2 concentration and airflow rate inside the chamber were maintained at ≈ 400 μmol mol^{-1} and 350 μmol s^{-1}, respectively. Sun-exposed fully expanded leaves (one-two leaves per tree and replicate for each treatment and cropping system, n = 4) were selected for measurements.

Canopy temperature (T_c) was real-time monitored using infra-red radiometer sensors (model SI-431 series, Apogee Instruments, Inc., Logan, UT, USA). Six sensors were installed for each cropping system, three per irrigation treatment (n = 3). Each sensor was mounted on a bracket attached to an aluminium pole, installed 6 m from the lime tree row, aimed at the middle south-facing side of the tree canopy (Fig. 2). The half field of view (FOV) of these sensors was 14°, with a response time of 0.1 s. The sensors were calibrated by the manufacturer, with an accuracy of ± 0.2 °C. They were connected to the cloud-based web server platform (sub-Section 2.2), which read T_c values every 5 min and recorded average values every 15 min

The following thermal-based indices were calculated throughout the experimental period:

(i) T_c –T_a: the difference between canopy and the surrounding air temperature.

(ii) CWSI (crop water stress index): calculated empirically according to the equation described by Idso et al. (1981):

$$CWSI = \frac{(T_c - T_a) - (T_c - T_a)LL}{(T_c - T_a)UL - (T_c - T_a)LL} \quad (1)$$

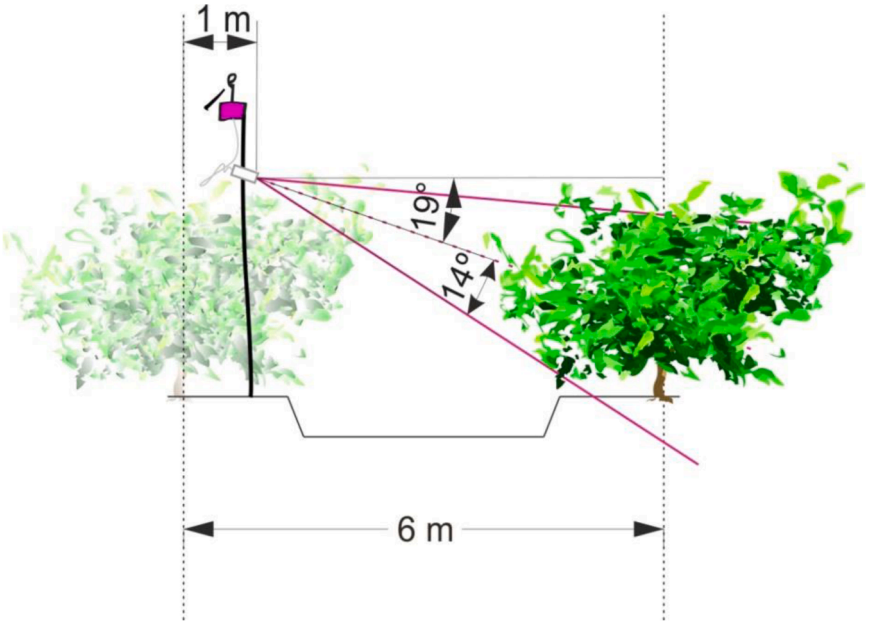

Fig. 2. Infra-red radiometer sensor placement.

where, T_c-T_a is the measured canopy to air temperature difference, (T_c-T_a)LL is the lower limit of (T_c-T_a) for a given vapour pressure deficit (VPD), named as the Non Water Stressed Baseline (NWSB), which is equivalent to a canopy transpiring at the potential rate, and (T_c-T_a)UL is the upper limit, which corresponds to a canopy where transpiration has ceased. The linear relationship between T_c-T_a and VPD for well irrigated trees (control treatment) was used to establish the NWSB for each cropping condition. (T_c-T_a)LL was calculated by solving the NWSB equation for the actual VPD. The (T_c-T_a)UL was a fixed value, taken from the maximum T_c-T_a value recorded in the stressed lime trees during the experiment. Only cloudless days were selected for the NWSB determination and CWSI calculation.

(iii) CTI* : a simplified canopy thermal index from that proposed by Mira-García et al. (2020):

$$CTI^* = \frac{(T_c - T_a)}{(T_c - T_a)max - \quad (T_c - T_a)min} \tag{2}$$

(iv) $\sum T_c$-T_a: a daily integral thermal index, for daylight values of T_c-$T_a > 0$.

2.4. Statistical analysis

The data were analysed by two-way analysis of variance (ANOVA) using SPSS statistical software, version 25 (IBM, Armonk, NY, USA), to discriminate between time and irrigation treatment–cropping system effects. Post hoc pair-wise comparison between all means was performed by the Least Significant Difference test at $p < 0.05$ ($LSD_{0.05}$). Linear regressions, Pearsońs correlation (R^2), and the mean squared error (MSE) were used to assess the significance of the relationships between the measured indicators.

3. Results

3.1. Soil-plant-atmosphere water status

During the experimental period, the reference crop evapotranspiration (ET_0) varied greatly from day to day, with higher values registered in open-field than in shaded conditions (Fig. 3A). The maximum daily ET_0 values were noted in mid-June (7.32 mm d^{-1}), and the minimum at the end of October (0.54 mm d^{-1}) in open-field conditions. Shaded condition reduced ET_0 by an average of 28 %, varying from the maximum of 4.30 to the minimum of 0.59 mm d^{-1} in June and October, respectively (Fig. 3A).

The discrete measurements of plant water status, on representative days during the experimental period, showed values of stem water potential (Ψ_{stem}) ranging from − 0.59 to − 1.19 MPa in control trees of both cropping systems (Fig. 3B). In turn, the real-time soil water content (θ_v), on the same control trees, showed in the active root zone (0.2–0.6 m), mean similar values in open-field (33.01 %) and in shaded conditions (32.22 %) (Fig. 3C). However, higher θ_v at 0.8 m was observed in shaded conditions as irrigation episodes were not observed to reach this depth in open-field conditions, or even was showed lower θ_v values (data not shown).

Withholding irrigation caused a gradual decline in soil and plant water status parameters (Fig. 3B, C). Ψ_{stem} values were statistically lower in stressed than in control trees from the beginning of the stress in both cropping systems (Fig. 3B). In the early stages of water stress, lower values of Ψ_{stem} were noted in open-field than in shaded conditions (Fig. 3B). Nevertheless, as the stress progressed, Ψ_{stem} continued to decrease in both cropping systems with not statistically significant differences between non-irrigated treatments ($S_{open\text{-}field}$ and S_{shaded}) (Fig. 3B).

An earlier and more abrupt decrease in θ_v was observed in non-irrigated open-field lime trees compared with shaded trees. The θ_v values were lower in stressed open-field lime trees up to mid-July, after

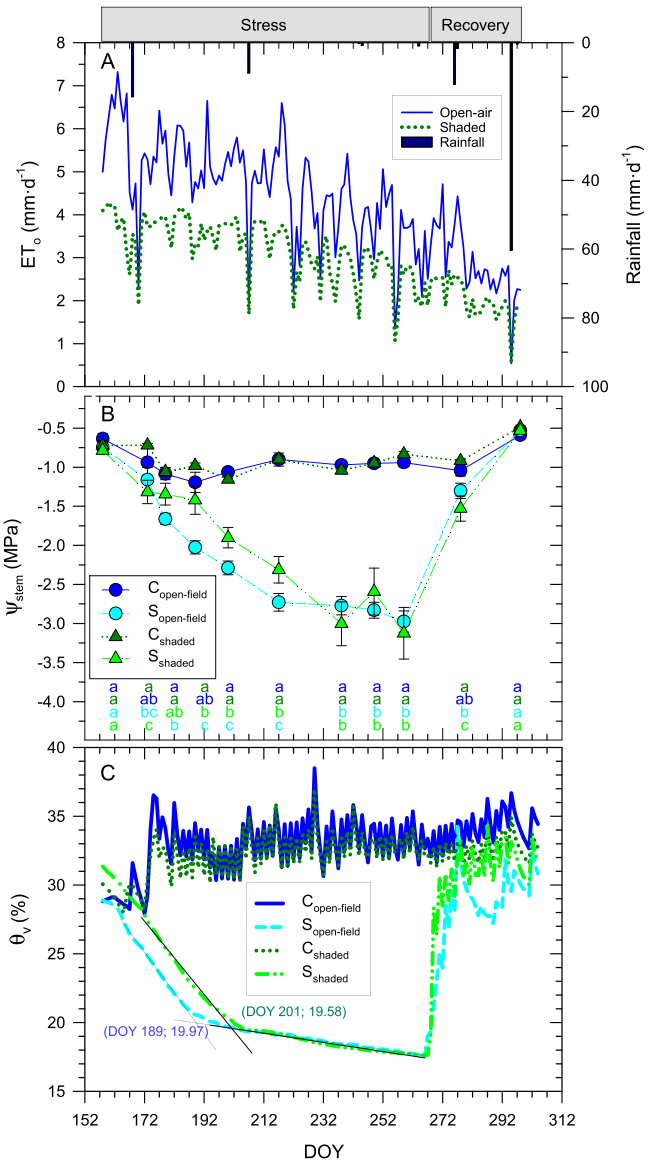

Fig. 3. Soil-plant-atmosphere system components during the stress and recovery periods: (A) daily reference crop evapotranspiration (ET_0) in open-field ( ) and shaded ( ) conditions, and rainfall ( ), (B) stem water potential (Ψ_{stem}), and (C) soil water content (θ_v), average of 0.2, 0.4 and 0.6 m soil depths, in control ( ) and stressed ( ) lime trees grown in open-field and shaded conditions, respectively. In (B), vertical bars on data points are standard error (± SE), and different letters indicate significant differences among treatments according to $LSD_{0.05}$ test. In (C), ∗ and ∧ indicate statistically significant differences between irrigation treatments in open-field and shaded conditions, respectively, according to $LSD_{0.05}$ test.

which the decrease was similar in both cropping systems (Fig. 3C). At the end of the stress period (DOY 267), minimum values were recorded for both water status indicators, with statistically similar values in open-field ($\theta_v = 17.5$ % and $\Psi_{stem} = -2.98$ MPa) and in shaded ($\theta_v = 17.6$ % and $\Psi_{stem} = -3.1$ MPa) (Fig. 3B, C) conditions.

In addition, two phases were detected in the soil drying curve of stressed lime trees, as drawn in Fig. 3C. A first sharp reduction in θ_v values was observed (phase I), followed by a slower rate of reduction (phase II). Phase I corresponded to DOY 173–189 in the case of open-field conditions, while it lasted from DOY 173–201 in shaded conditions. During this phase, θ_v decreased by ≈ 30 % in both cropping systems. In phase II, which lasted from the end of phase I to the end of stress

period: DOY 189–259, and DOY 201–259, in open-field and shaded conditions, respectively, θ_v decreased at a slower rate than during phase I. The breaking point of these two phases occurred earlier in open-field than in shaded conditions, but at a similar θ_v value (≈ 20 %) in both cropping systems (Fig. 3C). This intersection point of θ_v matched a similar value of $\Psi_{stem} \approx -2$ MPa, which was reached earlier in open-field than in shaded lime trees (Fig. 3B).

During the recovery period, when the water restrictions ended and the stressed trees were irrigated the same as control trees (from DOY 259–298), a gradual increase in both θ_v and Ψ_{stem} values was observed. Similar values of θ_v were noted in stressed and control trees 15 days after re-irrigation (Fig. 3C), whereas Ψ_{stem} recovered control values after one month, in both cropping systems (Fig. 3B).

3.2. Leaf gas exchange

Leaf gas exchange measurement in well irrigated trees showed high values of stomatal conductance (g_s), and net photosynthesis (P_n) (Fig. 4). Shade screen reduced the incident photosynthetically active radiation (PAR) by 22–47 % compared with the open-field conditions, with the maximum reduction observed in July-August corresponding to the months of highest evaporative demanding (data not shown). A similar seasonally fluctuating trend was noted in g_s and P_n values in the control trees in both cropping systems. Nevertheless, average values were significantly higher in shaded than in open-field conditions (Fig. 4), especially apparent for g_s, with a seasonal value of 105.5 and 122.3 mmol m^{-2} s^{-1} in open-field and shaded control trees, respectively. However, similar average values of P_n (11.1 and 10.6 μmol m^{-2} s^{-1}) were noted in open-field and shaded trees, respectively.

When the water stress was applied, the leaf gas exchange parameters gradually fell in both cropping systems (Fig. 4). Values of g_s in the control and stressed treatments statistically differed when irrigation began to be withheld in both cropping systems (Fig. 4A). In the early stages of water stress, a greater decrease in g_s and P_n was noted in the

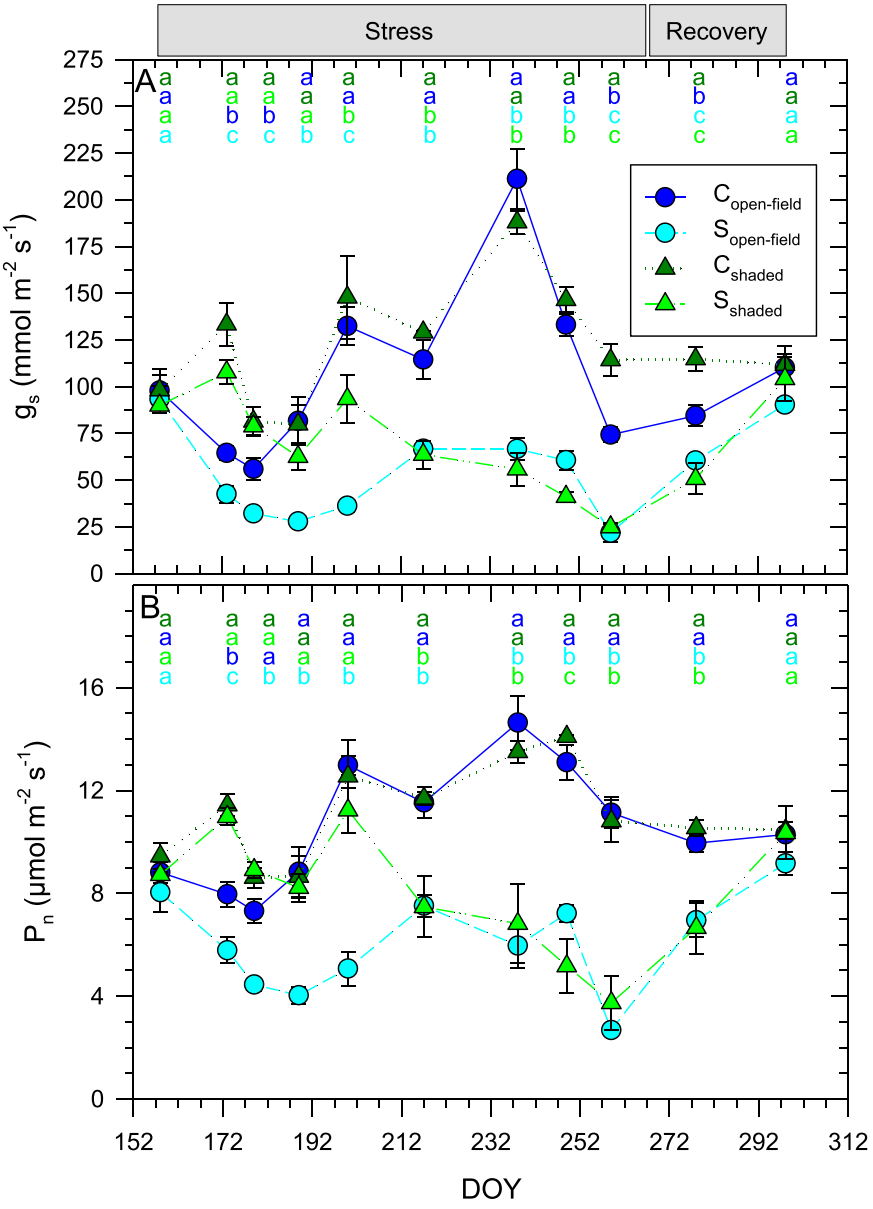

Fig. 4. Leaf gas exchange values during the stress and recovery periods: (A) stomatal conductance (g_s), and (B) net photosynthesis (P_n), in control (—●—, ▲) and stressed (—●—, —▲—) lime trees grown in open-field and shaded conditions, respectively. Vertical bars on data points are standard error (± SE). Different letters indicate significant differences among treatments according to $LSD_{0.05}$ test.

limes trees cultivated in open-field conditions. However, it should be noticed that P_n values in stressed trees cultivated in shaded conditions remained similar to control trees values for up to 40 days after irrigation was withheld (Fig. 4B). Average g_s values during the stress period were significantly lower in stressed lime trees grown in open-field than in shaded conditions (54.5 and 70.3 mmol m^{-2} s^{-1}, respectively). In addition, the same trend was observed in the case of P_n, with values of 6.1 and 8.0 μmol m^{-2} s^{-1}, for the trees cultivated in open-field and

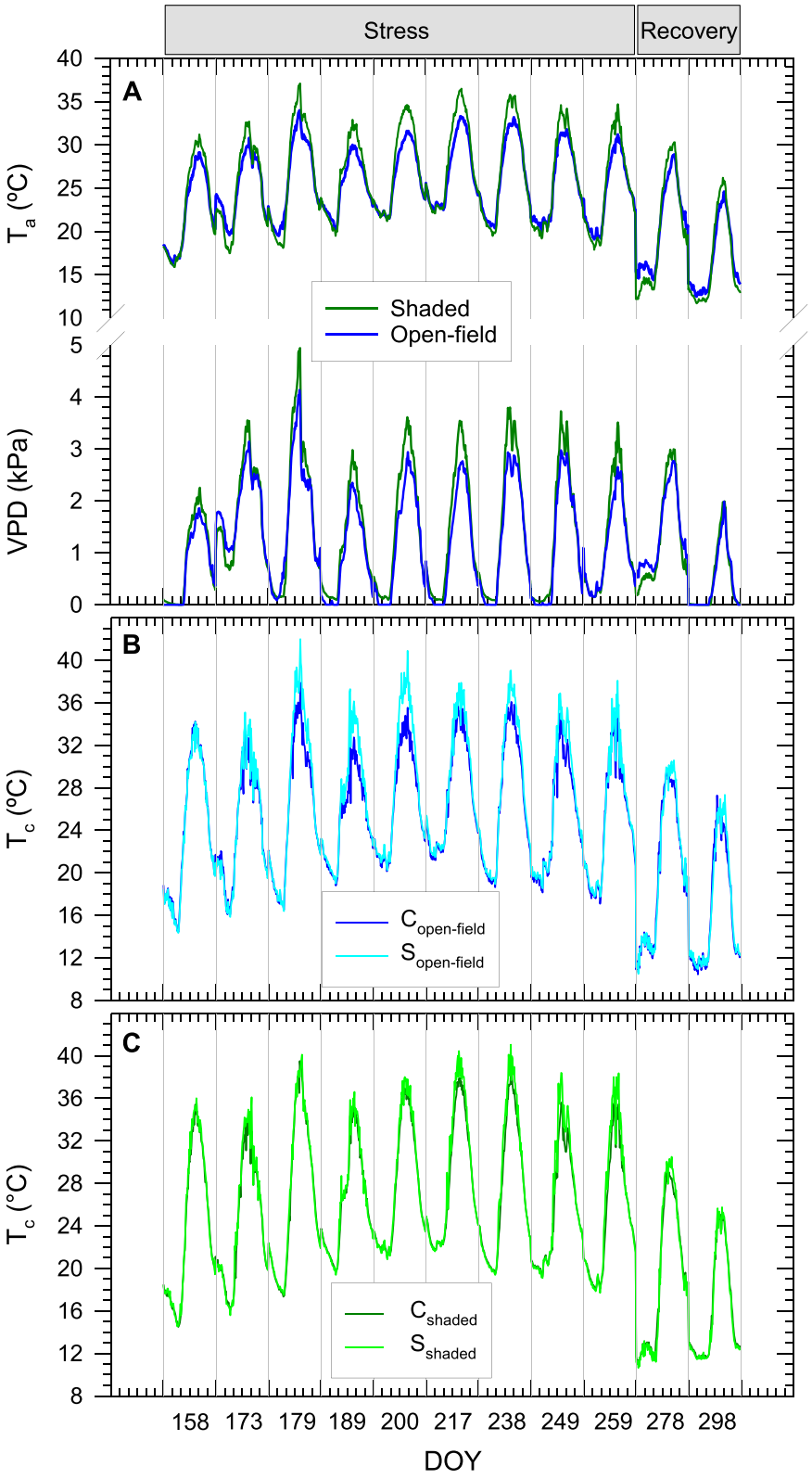

Fig. 5. Daily trend on selected days of (A) vapour pressure deficit (VPD) and air temperature (T_a) in open–field ( ) and shaded ( ) conditions; and (B and C) canopy temperature (T_c) in control (,) and stressed (,) lime trees grown in open-field and in shaded conditions, respectively. Data correspond to 15-min average values.

under shade screen, respectively. At the end of the stress period, leaf gas exchange values were low in the stressed lime trees, with no significant differences between cropping systems, $g_s \approx 23$ mmol m^{-2} s^{-1} and $P_n \approx 3.2$ μmol m^{-2} s^{-1} (Fig. 4). When the water stress period finished and all the trees were fully irrigated, g_s and P_n gradually increased, reaching similar values to those of the control trees after one month (Fig. 4).

3.3. Canopy temperature and thermal-based indices

During the experimental period, real-time canopy temperature (T_c) revealed widely varying daily values, with lower values recorded during night periods (always > 10 °C in both cropping systems and irrigation treatments) and higher during light-hours. The maximum T_c values were 44.5 and 45.7 °C in the control trees grown in open-field and shaded conditions, respectively, and up to 48 °C in both stress treatments (data not shown). Fig. 5 shows T_c and ambient conditions (T_a and VPD) for the days when Ψ_{stem} and leaf gas exchange were measured. The daily dynamics of VPD and T_a values revealed higher values in the atmosphere surrounding the lime trees grown in shade screen than in open-field conditions during the daytime. Nevertheless, during the night, the opposite was noted (Fig. 5A). It was clear that for both irrigation treatments and cropping systems, the night-time T_c values remained above 14 °C during the stress period and decreased to 10 °C during the recovery period. Maximum daily T_c values were observed at midday (around 12:00–14:00 GMT+2), coinciding with the highest daily VPD and T_a values (Fig. 5A). The greatest differences between irrigation treatments and cropping systems were detected at this time of the day, with T_c being higher in lime trees grown in shaded (Fig. 5C) than in open-field conditions (Fig. 5B). For instance, on DOY 179 and 200, T_c exceeded 40 °C in open-field lime trees, while the same occurred on four days in shaded conditions.

Daylight T_c was higher in stressed than in control trees, especially at midday, when differences were up to 6 and 5 °C, in open-field (Fig. 5B) and shaded conditions (Fig. 5C), respectively. This was more evident from DOY 173 and 189 onwards in open-field and shaded conditions, respectively. T_c in stressed trees remained above control values during the greatest part of the day and night hours in open-field trees. However, in shaded conditions stressed trees showed similar or even lower values of T_c than control trees during night-hours. During the recovery period, T_c as well as T_a and VPD values were lower than during the stress period, and the T_c of the stress treatments recovered to reach similar values to those of the control at the end of the experimental period (DOY 298).

To normalize the absolute value of T_c respect to T_a, several thermal-based indices (T_c-T_a, CWSI, CTI* and $\sum T_c$-T_a) were calculated throughout the experimental period (stress and recovery) in the different irrigation treatments and cropping systems studied. The canopy to air temperature difference (T_c-T_a) varied on a diurnal basis (Fig. 6) but to a lesser extent than T_c (Fig. 5B, C). Canopy temperature remained below T_a during most of the daylight hours, with negative T_c-T_a values corresponding to night periods. Independently of the cropping systems, the highest T_c-T_a values were observed in stressed trees (Fig. 6C, E).

Furthermore, higher values of this thermal index were recorded in open-field (Fig. 6B, C) than in shaded conditions (Fig. 6D, E), again independently of the irrigation treatment. Average 15-min values of T_c-T_a during the experimental period was negative in control trees (−0.2 °C, in both cropping systems) and positive in stressed trees (0.6 and 0.2 °C, in open-air and shaded conditions, respectively). It can also be observed in Fig. 6, that daily maximum T_c-T_a in the control trees varied around average value of 4.3 and 2.6 °C, in open-field and shaded conditions, respectively, while they were 6.3 and 4.4 °C in the stress treatments, in open-field and shaded conditions, respectively (as outlined in Fig. 6B, C and D, F, respectively). Moreover, wider fluctuations in daily T_c-T_a values were noted in stressed trees (Fig. 6C, E) than in controls trees (Fig. 6B, D).

The crop water stress index (CWSI) was calculated using Eq. 1 for each cropping system, with the lower limit taken from the T_c-T_a *vs.* VPD linear relation (named NWSB) of well irrigated lime trees. In the analysis of all 15-min data, we noted a wide hysteresis with higher T_c-T_a in the morning (denoting warming) and lower in the afternoon (denoting cooling). Considering only daylight values, differences remained depending on the time interval. Positive slopes of the linear regression (values of 0.22 and 0.10, for open-field and shaded conditions, respectively) were found around midday (12:00–14:00 GTM+2).

The NWSB was then selected from the best regression, which corresponded to the time interval 09:00–10:00 GMT+ 2 for open-field trees (T_c-$T_a = -0.90 \cdot VPD + 1.52$, $R^2 = 0.77$**), while the corresponding time interval selected for shaded trees was 10:00–12:00 h GMT+ 2 (T_c-T_a

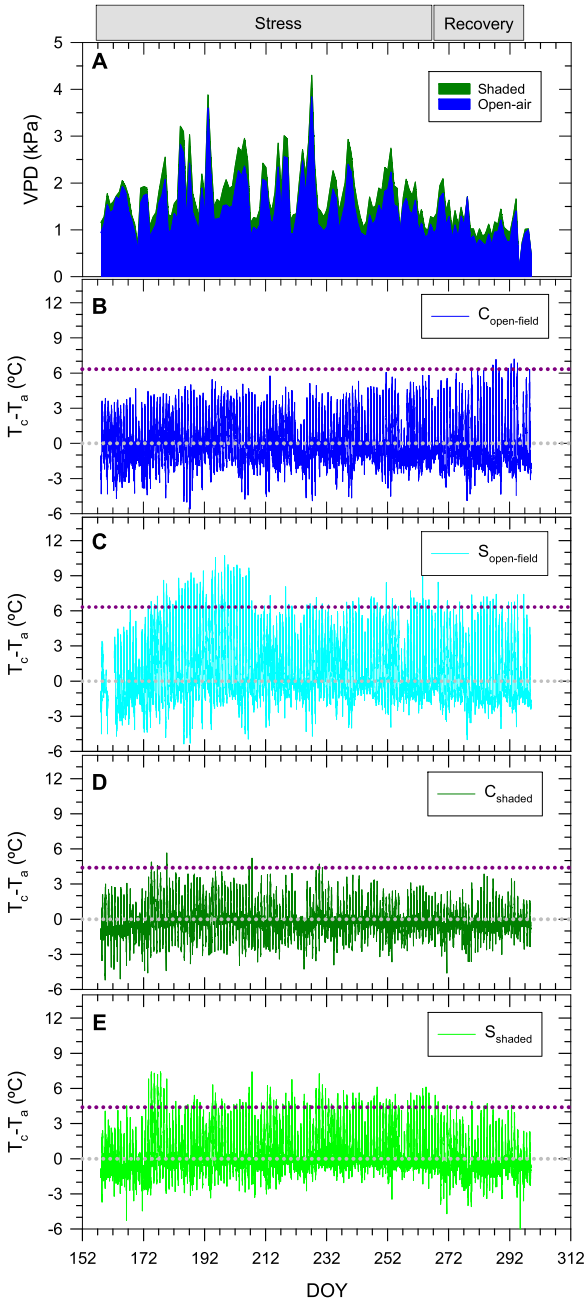

Fig. 6. Time courses during the stress and the recovery periods of (A) average daily vapour pressure deficit (VPD) in open-field ( ) and shaded ( ) conditions; and canopy to air temperature difference (T_c-T_a) in control (,) (B and D) and stressed (,) **(C and E)** lime trees grown in open-field and shaded conditions, respectively. T_c-T_a data corresponds to 15-min average values.

$= -0.83 \cdot VPD + 0.85$, $R^2 = 0.92$***). The covariance analysis revealed no significant differences among the slopes, and so one regression was considered for the two cropping systems (T_c-$T_a = -1.05 \cdot VPD + 1.57$, $R^2 = 0.91$***, MSE = 0.13).

In an attempt to obtain the lower limit, the T_c-T_a *vs*. VPD regressions for stressed lime trees were drawn using data from DOY 200 onwards (last part of the stress period). They revealed quasi-parallel lines to the VPD axis, with very low slopes for both cropping systems (data not shown). The intercept on the T_c-T_a axis varied during the day, as can be observed in Fig. 7, increasing from the beginning of daylight hours and reaching maximum values around midday. At this time of the day, higher values were observed in open-field than in shaded trees (≈ 6 and 4 °C, respectively), showing a delay of about 2 h in the case of shaded trees (Fig. 7). Thus, the upper limit was selected for the same time interval as that for which the lower limit was calculated, 09:00–10:00 GMT+ 2 for open-field (1.51 °C), and 10:00–12:00 h GMT+ 2 for shaded conditions (1.53 °C). Note that these values were similar to that of the intercept for the above described NWSB (1.57 °C).

The CWSI values were higher in open-field (Fig. 8A) than in shaded conditions (Fig. 8B) in control treatment, while no-significant differences were observed between stressed treatments. In open-field conditions, the differences between stress and control treatment were apparent after one month of stress, with maximum CWSI values observed by mid-July (DOY 200) in both irrigation treatments, reaching values around 1 in stressed lime trees. After this time, the CWSI of stressed trees remained ≈ 0.1 higher than in the control trees, but with no-significant differences, during most of the irrigation withholding period (Fig. 8A). Nevertheless, in shaded conditions the differences between both irrigation treatments were minimal during the early stress period, and gradually increased, being statistically significant higher as stress accumulated, with maximum CWSI values (close to 1) being reached at the end of the stress period (Fig. 8B). In the recovery period, the CWSI decreased to 0.3 in both cropping systems (Fig. 8A, B), values even lower than their respective control treatments.

The integration of T_c-T_a values on a daylight time basis suggested a new thermal-based index: $\sum T_c$-T_a. From the beginning of the experiment $\sum T_c$-T_a gradually increased in all cropping systems and irrigation treatments (Fig. 9A). At the end of the experimental period, the fully irrigated trees showed values of 2057 and 933 °C in open-field and shaded conditions, respectively. Nevertheless, the stressed plants showed 2119 and 1105 °C greater than their control, in open-field and shaded conditions, respectively. The differences between control and stress treatment were evident by the end of June (DOY 179) in open-field trees, and several days later in shaded trees. At the end of the experimental period, stressed shaded lime trees showed similar values to those of the control trees in open-field condition (Fig. 9A).

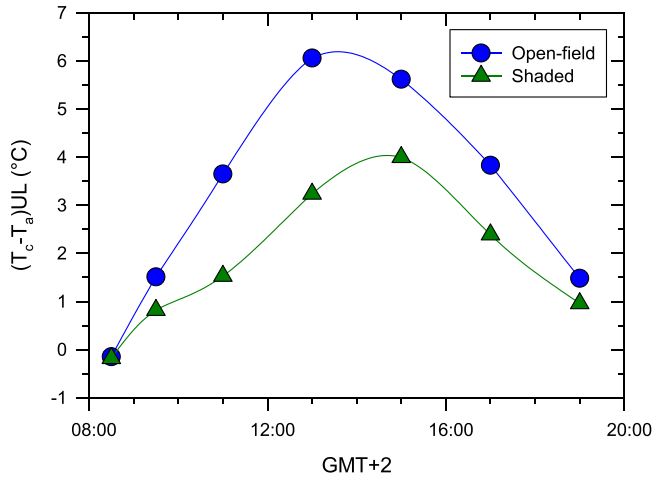

Fig. 7. Intercept of the T_c-T_a *vs*. VPD relationship for the different diurnal time intervals (GMT+2) in the stressed lime trees grown in open-field (—●—) and in shaded (▲) conditions.

Furthermore, T_c-T_a values were normalized by dividing by the difference between maximum and minimum daily value (Eq. 2). At the beginning of the experiment (DOY 158), the trees cultivated inside the shade screen house showed lower CTI* values (0.6 and 1.1 to control and stress treatments, respectively) than the trees cultivated in open-field conditions (2.0 and 1.5 to control and stress treatments, respectively). Differences between control and stress treatments occurred earlier and reached higher values in open-field than in shaded conditions (Fig. 9B). At the end of the recovery period, similar values of CTI* were noted in control and stress trees grown in shaded conditions (0.6 and 0.7, respectively). Nevertheless, slightly different values were observed in control and stress trees cultivated in open-field conditions (1.9 and 2.7, respectively).

For both cropping systems a linear regression model was made between the punctual measurements of Ψ_{stem} and leaf gas exchange and the continuous measurements of thermal indexes and θ_v. Only those between Ψ_{stem} and T_c-T_a are presented as they are the most relevant (Fig. 10). Values of T_c-T_a were averaged for the time period when the stem water potential was measured, around solar noon (12:00–14:00 GMT+2), which coincided with maximum daily evaporative demand conditions. The relationship between T_c-T_a and Ψ_{stem} showed good correlation regardless of the cropping system, with R^2 fits of 0.57** and 0.55** in open-field and shaded conditions, respectively. Also of note were the similar negative slopes (≈ −1) in both cropping systems. This linear correlation between both plant water status indicators, although empirical, can be used as a guide for irrigation management. Therefore, if the objective is to maintain the lime trees without stress, the Ψ_{stem} should be − 1.0 MPa, and, by solving this equation (Fig. 10), T_c-T_a would correspond to 2.4 or 0.8 °C for open-field and shaded lime trees, respectively. For a moderate water deficit condition ($\Psi_{stem} = -1.5$ MPa), the T_c-T_a would correspond to 2.9 or 1.3 °C, while for severe water stress conditions ($\Psi_{stem} = -2.0$ MPa), the T_c-T_a would be 3.4 or 1.83 °C, for open-field and shaded lime trees, respectively.

4. Discussion

During the experimental period, the environmental conditions were characteristic of Mediterranean climates, with high ET_0 and VPD values in the summer months, coinciding with the stress period (DOY 158–267), and low values during autumn, in the recovery period (DOY 267–298). The microclimate surrounding lime trees differed slightly in both cropping systems, PAR radiation being lower under shade screen than in open-field (Fig. 3A). This would be a consequence of the screening increasing light scattering (Nissim-Levi et al., 2008), leading to a decrease in the incoming radiation (Ombódi et al., 2015). This reduction depends, amongst other factors, on the screen type, colour, and percentage of shading (Mditshwa et al., 2019). In orange and in avocado trees, Incesu et al. (2016) and Tinyane et al. (2018), respectively, observed a reduction of PAR radiation ranging from 9 % to 46 % depending of colour and % shading. In our study reductions in PAR were seasonal in character, pronounced reductions coinciding with summer and the lower reductions with autumn, as was found by Zhou et al. (2018) and Mira-García et al. (2020) in shaded *Citrus* trees. This seasonal character could be justified by a change in the solar inclination over the year. The reduction in incoming PAR radiation observed under netting smoothed the ET_0 values, an observation lent weight by Jagadale et al. (2018), who observed reductions in the ET_0 of 12–24 %, depending on the colour of the shading screen. In addition, the smoother values of ET_0 might have been the consequence of reduced wind speed observed in this cropping system. Similarly, Blakey et al. (2016) and Wachsmann et al. (2014) observed a reduction in wind speed of up to 85–90%, in avocado and mandarin trees, respectively grown under screening conditions.

The evaluation of the ability of discrete (Ψ_{stem}, g_s and P_n) and

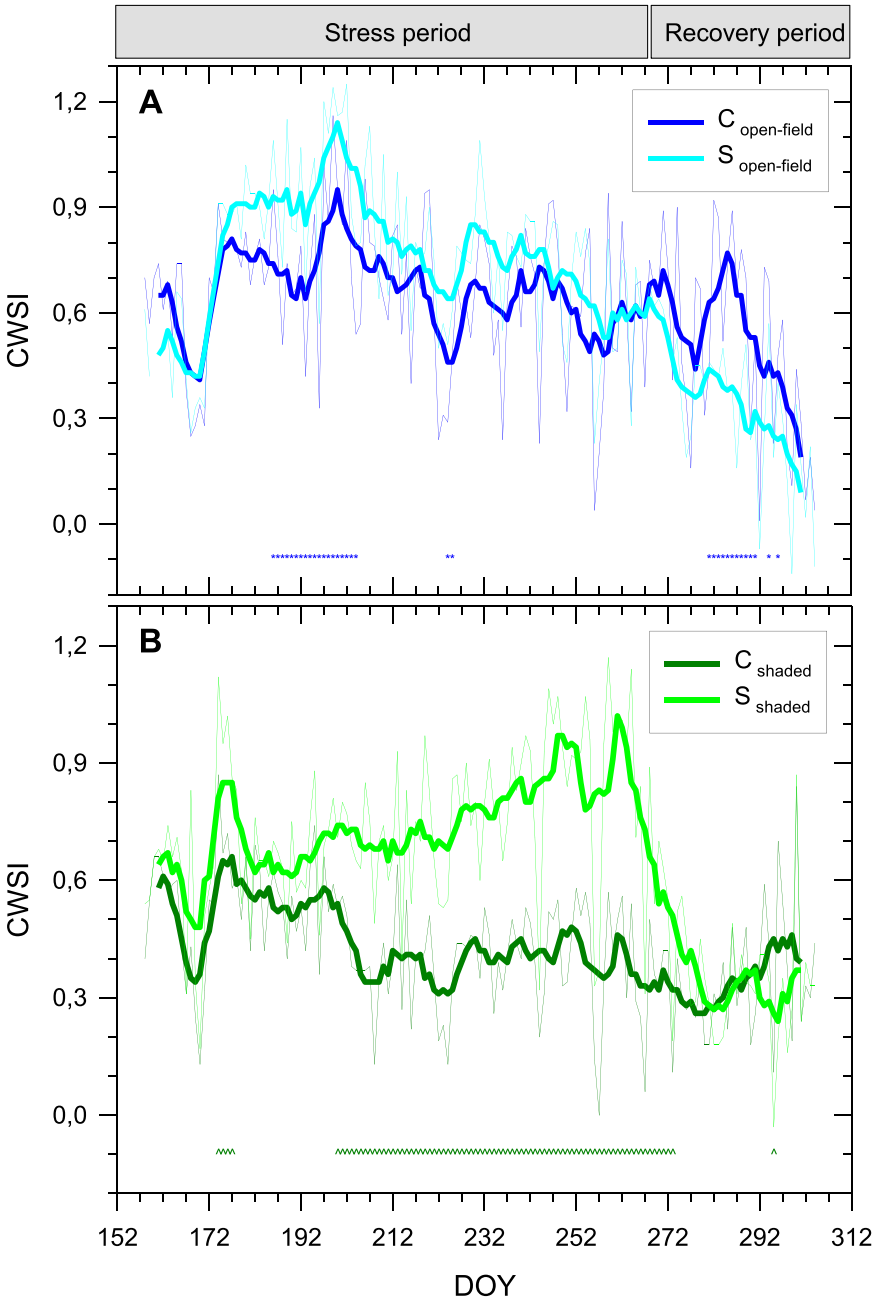

Fig. 8. Crop water stress index (CWSI) during the stress and recovery periods: in control (– , –) and stressed (– , –) lime trees grown in (A) open-field and in (B) shaded conditions, respectively. Data correspond to daily average values and bold lines to seven-day moving average. ∗ and ∧ indicate statistically significant differences between irrigation treatments in open-field and shaded conditions, respectively, for daily values, according to $LSD_{0.05}$ test.

continuous plant (T_c) and soil (θ_v) water status indicators to detect water stress revealed Ψ_{stem} as a reliable water status indicator, greatly affected by the soil water content and in a lesser extent by shading conditions. The well-irrigated lime trees showed an optimal soil water status in both cropping systems, as revealed by the high θ_v values in the active root zone, which varied around field capacity values (Fig. 3C). The observed fluctuations in θ_v would have been due to soil wetting /drying cycles corresponding to irrigation events and the periods between them. In the experimental period of our study, automated irrigation was carried out daily or every other day, as is normal for drip irrigation scheduling for *Citrus* during the summer months in Mediterranean climates. In addition, midday Ψ_{stem} values in control lime trees pointed to the absence of a water stress situation, with values > -1 MPa, which is indicative of an optimal water status for *Citrus* species, mentioned in several studies (Ballester et al., 2014; Conesa et al., 2018; Pagán et al., 2012), and there was no clear influence of the cropping system on Ψ_{stem} dynamics (Fig. 3B).

Withholding irrigation water decreased the soil water status (Fig. 3C) from the beginning of water stress. The plant water status gradually fell in stressed lime trees as θ_v values decreased, up to Ψ_{stem} values (Fig. 3B), similar to that found in non-irrigated grapefruit trees (Pérez-Pérez et al., 2009) but lower than the levels measured in deficit-irrigated sweet orange trees (Gasque et al., 2016). When the water stress was moderate, the stress was affected by the cropping system, the decrease soil and plant water status being earlier and more rapid in open-field than in shaded conditions. Nevertheless, when the water stress was severe, the decrease of soil and plant water status was similar for both cropping systems. Proof of this is that stressed lime trees reached a Ψ_{stem} value of

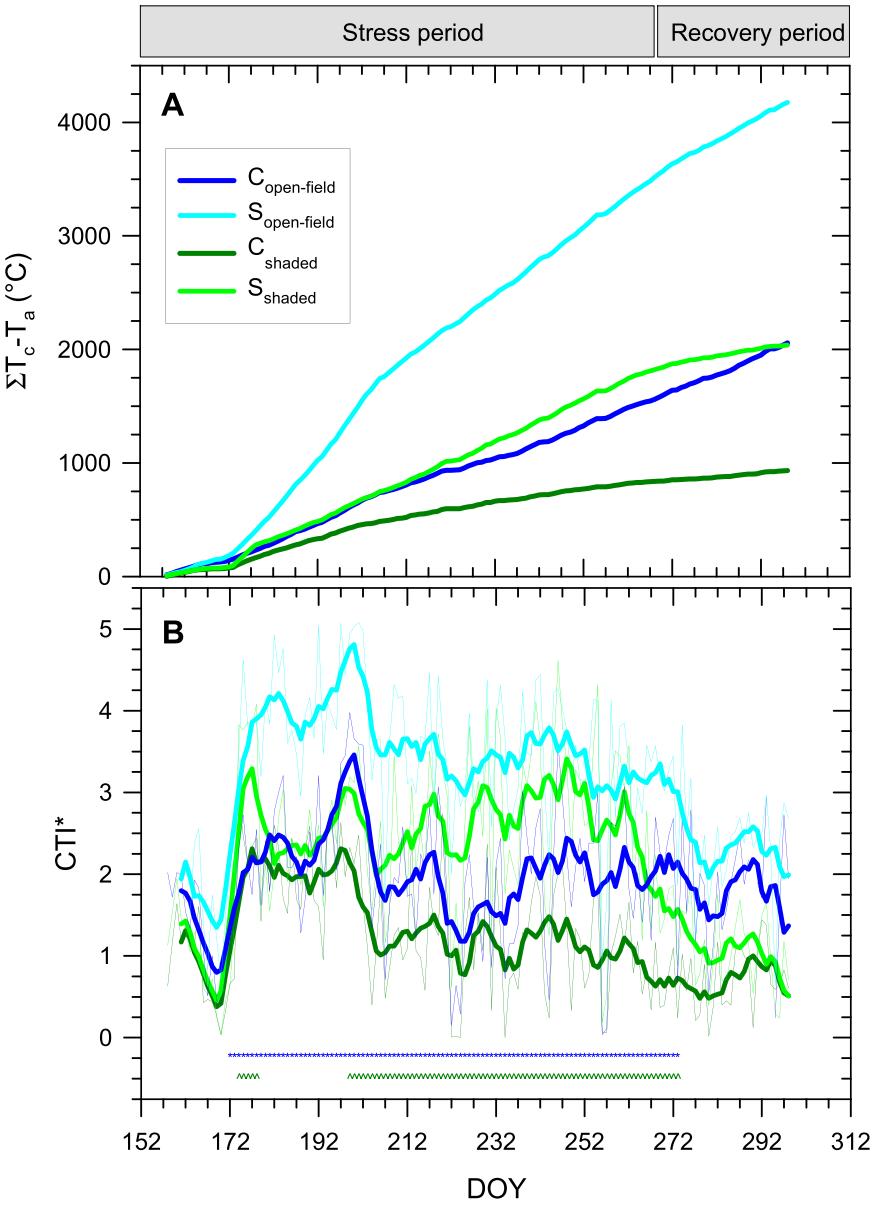

Fig. 9. Time course of **(A)** Integrated daylight $\sum T_c$-T_a, and **(B)** Canopy thermal index (CTI*) during the stress and recovery periods in control (– , –) and stressed (– , –) lime trees grown in open-field and shaded conditions, respectively. In (B), data correspond to daily average values and bold lines to seven-day moving average. ∗ and ∧ indicate statistically significant differences between irrigation treatments in open-field and shaded conditions, respectively, for daily values, according to $LSD_{0.05}$ test.

about − 2.0 MPa one month after beginning to withhold irrigation in open-field conditions and 15 days later in shaded conditions (Fig. 3B). When this Ψ_{stem} value was reached, the transition from a relatively rapid reduction in the soil water content slowed down in both stress treatments (Fig. 3C). Known as the 'breaking point', this could be used as an indicator of the beginning of severe water stress in lime trees, allowing critical irrigation monitoring. It must be pointed out that when the experiment started, shaded and open-field had different SWC values, as a consequence of the different microclimatic conditions in the two cropping systems (Fig. 3A and 5A). As a result, the breaking point date delayed in shaded compared with open-field conditions, which is consistent with the root water uptake rate of both cropping conditions. Similarly, the change in the day-time reductions of θ_v from a sharp decrease to a slower rate (Girona et al., 2002) has been suggested as representing the beginning of stress in *Prunus* (Abrisqueta et al., 2012) and vegetable crops (Thompson et al., 2007). In the recovery period, when stressed trees were re-irrigated, Ψ_{stem} gradually recovered to reach control values, indicating that plant water status is a true reflection of soil water availability, although also dependent on meteorological conditions (Suter et al., 2019).

Withholding irrigation for 110 days in our study induced statistically lower average Ψ_{stem} values in stressed lime trees grown in open-field (−1.96 MPa) than in shaded (−1.78 MPa) conditions. However, similar Ψ_{stem} values (≈ −3.0 MPa) were observed in both cropping systems at the end of the stress period (Fig. 3B), which is considered a severe water stress situation for perennial fruit trees (Hsiao, 1990).

The leaf gas exchange in control lime trees showed greater day-to-day variability (Fig. 4), but with the characteristic values of well-watered trees (Mira-García et al., 2021). These fluctuations were greater than those observed in θ_v and Ψ_{stem} (Fig. 3B,C), indicating that, apart from soil water status, the leaf gas exchange was influenced by other factors, including ambient conditions and changes in the internal ABA concentration (Rodriguez-Dominguez et al., 2019). The high g_s and P_n values observed coincided with the period of active lime fruit growth, as was observed in grapefruit (Romero-Trigueros et al., 2014) and lemon (García-Orellana et al., 2013) trees. This fact, along with the decreasing trend in leaf gas exchange during the recovery period (note that harvest occurred by mid-August (first pick) and end of September (second

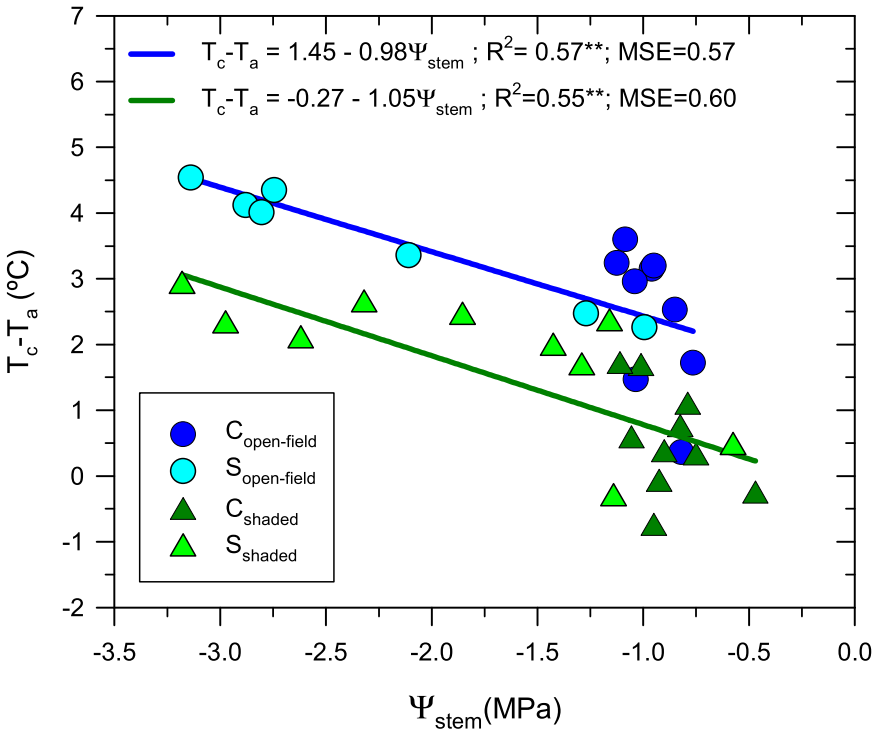

Fig. 10. Relationship between Ψ_{stem} and $T_c - T_a$ in in control (●/▲) and stressed (○/△) lime trees grown in open-field and in shaded conditions, respectively. $T_c - T_a$ values corresponded with the average measurements taken between 12:00 and 14:00 GMT+ 2. Linear regression and R^2 included in the graph. MSE means mean squared error.

pick)), was attributed to the feedback effect of the actively growing fruits on leaf photosynthesis (Ben Mimoun et al., 1996).

Stomatal aperture in fully irrigated trees was lower in open-field than in shaded conditions (Fig. 4A), perhaps because the milder ET_0 (Fig. 3A) and PAR conditions under shading favoured stomatal aperture and photosynthetic capacity, as observed in shaded *Citrus* sp. (Alarcón et al., 2006; Mira-García et al., 2021). Likewise, De Freitas et al. (2013) observed a 56 % increase in g_s and Bastías et al. (2012) a 28 % increase in P_n in apple trees grown under black net (50 % shading) and a blue photo-selective net, respectively.

During the early stages of the water stress period, the lime trees acted normally in terms of leaf gas exchange. Nevertheless, when the plant water stress was moderate stomatal closure was favoured in open-field conditions (Fig. 4A). As stress progressed and Ψ_{stem} continued to decrease (Fig. 3B) stomatal conductance (Fig. 4A) and net photosynthesis (Fig. 4B) in both cropping systems reached values similar to those noted in *Citrus* sp. subjected to water deficits (García-Orellana et al., 2007; Pérez-Pérez et al., 2009). This indicates that, when the soil water content was very low, shade screening was not sufficient to mitigate the negative effect of water stress on its own, as Lopez et al. (2018) suggested. Moreover, an asynchrony effect between g_s and P_n was detected in the response to water stress, P_n decreasing (Fig. 4B) later than g_s in the stressed lime trees (Fig. 4A). This suggests that stomatal closure prevents the outflow of water vapour to a greater extent than the inflow of CO_2.

Since a decrease in transpiration due to stomatal closure causes the canopy temperature (T_c) to increase (as the so-called "evaporative cooling "decreases), T_c has long been recognized as an indicator of the plant water status (Gates, 1964). The development of infra-red sensors that measure infra-red radiation, which can determine surface temperature without touching it, has increased its use as an indicator of the degree of plant water stress related to water availability (Costa et al., 2013; Jones, 2004). In all the lime trees, T_c values varied greatly, following marked circadian rhythms, with high values during midday hours (also higher in stressed than in control trees) and low at night (Fig. 5). Such fluctuations in T_c could be due to the wide variations of g_s in the diurnal cycle, as observed by Sánchez-Blanco et al. (1990), who found that g_s values in field-grown lemon trees changed every half hour.

The evaluation of the response of different thermal indexes (T_c-T_a, CWSI, CTI* and ΣT_c-T_a) to irrigation and shade screen allowed to identify the best performing thermal-based index. The results showed that the daily course of T_c-T_a also varied, but to a lesser extent than T_c. The T_c-T_a dynamics throughout the experimental period could be likened to the "heartbeat" of lime tree water status, with higher values monitored in stressed than in control trees (Fig. 6). Negative T_c-T_a values indicate that an evaporative cooling process is occurring, i.e. heat is dissipated by the water lost from the stomata via transpiration, but as soon as stomata close, the canopy temperature raise several degrees above the air temperature (Araus et al., 2008; Jones et al., 2002; Lima et al., 2016). It is clear that there were no differences in T_c-T_a values between control and stress treatments in the absence of solar heating (from approx. 20:00 h to 7:00 h the next morning). In spite of the higher T_c values in the lime trees grown under shade screening (Fig. 5B, C), the T_c-T_a values were lower in shaded conditions than in open-field trees (Fig. 6), since the T_a was higher under screen (Fig. 5A), as has been observed in other studies (Alaphilippe et al., 2016; Kim et al., 2022). This anti-intuitive situation denoted that shaded trees were better paired with the environmental conditions they were in, which also led to lower T_c-T_a values for stressed trees compared with the stressed open-field trees (Fig. 6).

The CWSI can be calculated by empirical or theoretical methods. Even though the theoretical method, based on the energy balance (Jackson, 1982), is more precise, its complexity in terms of data collection and calculation, has meant that the empirical method, which is based on equations to determine the lower and upper limits of canopy

temperature, has tended to be favoured. The lower limit is derived from the linear equation between the VPD and T_c-T_a in well-watered conditions, while the upper limit is considered a constant value from non-transpiring canopies (Idso et al., 1981). Many authors have used a constant as the upper limit, assuming that canopy is always above air temperature when transpiration ceases, for example T_a + 4 °C, in grapefruit trees (Romero-Trigueros et al., 2019), or T_a + 5 °C in olive trees (Agam et al., 2013). Also, the upper limit could be assumed from the intercept of the NWSB corrected for T_a (González-Dugo et al., 2014) or from the maximum measured T_c-T_a value (Irmak et al., 2000; Yang et al., 2021). In our study, the lower limit was derived from the linear equation between the VPD and T_c-T_a in well-watered lime trees, although it resulted highly time-dependent, and the maximum T_c-T_a difference measured under severe water stress conditions was fixed as the upper limit (Fig. 7), which also coincided with that value assumed by the intercept of the NWSB, confirming the versatility of both methods.

We found that CWSI calculations were too empirical and too time interval-dependant, with wide day-to-day variations (Fig. 8). Other proposed thermal indices, based on absolute T_c-T_a values, were used as a reliable way to monitor real-time plant water status: ΣT_c-T_a (Fig. 9A) and CTI*(Fig. 9B). CTI*, compared to CWSI, better discriminated differences between irrigation treatments and cropping conditions. In our study, the cropping system and irrigation treatment had a clear effect on both thermal-based indices, in such a way that higher values of ΣT_c-T_a and CTI* were observed in open-field than in shaded conditions, and in stressed than control trees. Along the experimental period, the highest values corresponding to stressed open-field trees (Fig. 9). This indicates that in this cropping system (open-field) the meteorological conditions were less favourable, inducing a poorer plant thermal water status (Mira-García et al., 2020). At the same time, higher values of both thermal indices were observed in stressed trees than in control trees of both cropping systems, confirming that restrictions in the soil water availability induce stomatal closure (Blanco et al., 2019; De la Rosa et al., 2014), thus increasing the values of the thermal indices.

Thermal point or thermal imagery data relationships with Ψ_{stem} and g_s have been suggested for irrigation scheduling in *Citrus* and fruit trees (Egea et al., 2017; González-Dugo et al., 2014; Romero-Trigueros et al., 2019; Zhang et al., 2017). However, apart from that of Sepaskhah and Kashefipour (1994) on sweet lime trees in Iran, to our knowledge no studies have been carried out in lime trees. In our study, the correlation between T_c-T_a and Ψ_{stem} to determine threshold thermal based values for irrigation scheduling, differed in open-field and shaded conditions. Nevertheless, a similar linear correlation (slope ≈ 1) was noted in the trees cultivated under screening and open-field conditions, but with a different intercept (Fig. 10). Similar lineal adjustment between T_c-T_a and Ψ_{stem} was found by Ballester et al. (2013a) in 'Clementina de Nules' cultivated in open-field conditions. The midday T_c-T_a threshold values proposed for triggering drip irrigation in lime trees grown in semi-arid conditions were 2.4 or 0.8 °C for open-field and shaded conditions, respectively, but only when irrigation water availability is sufficient to fulfil plant water requirements. Under deficit irrigation conditions, the proposed values were 2.9 or 1.3 °C, for moderate water deficits (Ψ_{stem} = −1.5 MPa) and 3.4 or 1.83 °C, for severe water deficits (Ψ_{stem} = −2 MPa), for open-field and shaded lime trees, respectively. Similarly, Tormann (1986) proposed two T_c-T_a threshold values for irrigation management of nectarine trees, based on two levels of plant water status (Ψ_{stem} values) - well irrigated and moderately stressed. According with our findings, shading conditions should be taken into account when seeking threshold values for irrigation management, tailoring guidelines to the needs of sustainable agriculture.

The statistically higher Ψ_{stem} and leaf gas exchange values and lower canopy to air temperature differences recorded in shaded than in open-field lime trees revealed that the shade screening allowed better soil-plant water status conditions. Furthermore, it can be assumed that water consumption by trees was lower, since the irrigation applied was the same in both crop conditions. Consequently, shade netting conditions allowed a window of opportunity of ≈ 12 days to postpone a water deficit situation with respect to open-field conditions. This could be the result of the improved water use efficiency induced by shade screening, as has been noted in several *Citrus* sp., including mandarin (Wachsmann et al., 2014), orange (Medina et al., 2002), lemon (Alarcón et al., 2006; Nicolás et al., 2008) and grapefruit (Jifon and Syvertsen, 2003) trees.

Early signs of a water deficit situation are desired. In this sense, the soil water content sensor, wrapped around by the plant roots (thus behaving rather like a physiological sensor), anticipated the lime tree water status situation respect to thermal-based indices. Nonetheless, both monitoring systems showed close agreement as regards the water deficit level throughout the experiment.

5. Conclusion

This study has revealed that the response of lime trees to soil water deficits differed in the two cropping systems studied (open-field and shaded) and that the ability to detect water stress also differed for the soil/plant water status indicators studied. The main conclusions can be summarised as: (i) Precise estimates of the soil-plant-atmosphere components are needed to improve irrigation management; (ii) Ψ_{stem} is a reliable tool for assessing lime tree water status and along with the breaking point of θ_v are useful for irrigation monitoring; (iii) The thermal-based indices serve as an alternative to other classical discrete physiological measurements for remotely monitoring lime tree water status, while the daily T_c-T_a dynamics reflects the "heartbeat" of lime trees; (iv) Soil water content sensors anticipate water stress detection better than thermal-based sensors; (v) The higher Ψ_{stem} g_s, P_n, and lower T_c-T_a values observed in shaded than in open-field lime trees reveals that shading allows a better soil-plant water status and lowers water consumption, thus delaying plant water stress; (vi) The cropping system must be considered to propose T_c-T_a threshold values for lime tree drip irrigation management.

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CRediT authorship contribution statement

Conceptualization and investigation, A. B. M.-G., W.C., J.V. and M.C. R.-S.; data curation, A. B. M.-G., J.V. and W.C.; writing—original draft preparation, A. B. M.-G., and M.C.R.-S.; writing—review and editing, A. B. M.-G., W.C., J.V. and M.C.R.-S.; supervision, project administration and funding acquisition M.C.R.-S. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

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ARTICLE IV:

Estimation of stomatal conductance by infrared thermometry in citrus trees cultivated under regulated deficit irrigation and reclaimed water

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Objectives

The aim of this study was to evaluate the robustness of infrared thermometry to estimate stomatal conductance (g_s) in grapefruit trees for different water sources, irrigation strategies and phenological stages. In addition, the ability of different thermal indices to determine g_s at different thermal data intervals, vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) conditions was tested.

Materials and Methods

The experiment was carried out in 2019-2020 in a 0.5 ha orchard at Murcia, Spain. The experimental plot was cultivated with 14-year-old ´Star Ruby´grapefruit trees (*Citrus paradisi* Macf.,) grafted onto *Citrus macrophylla* L. rootstock and planted at 6 m x 4 m. The irrigation system consisted of a single lateral drip line with four pressure-compensated emitters of 4 L h^{-1} per tree. Irrigation was scheduled according to crop evapotranspiration (ET_c) accumulated during the previous week.

The robustness of infrared thermometry to estimate g_s was assessed for different i) water sources: transfer water (TW, optimal for agricultural uses) and saline reclaimed water (RW), ii) irrigation strategies: full irrigation (FI) and regulated deficit irrigation (RDI), and iii) phenological stages: flowering-sprouting (FS) and fruit growth (FG). FI trees were irrigated at 100 % of ET_c during the experimental period. Trees in RDI regime were also irrigated at 100 % of ET_c except in the phase II of fruit growth, when they were irrigated at 50 % of ET_c. In this study, correlations of canopy temperature (T_c), canopy to air temperature difference (T_c-T_a) and crop water stress index (CWSI) *vs* g_s were tested under several thermal data average intervals (60 min and timepoint), VPD ($VPD_{\leq 2}$ and VPD_{global}) and PAR (PAR_{1200} and $PAR_{>1200}$) conditions.

Meteorological conditions were recorded in real time from a weather station located at the experimental site. The g_s was punctually measured with a portable photosynthesis system and T_c was continuously monitored by infrared sensors. The average values of T_c were normalised by calculating the T_c-T_a and CWSI indices.

Results and Discussion

As for water quality, TW-FI trees showed higher g_s than RW-FI trees, due to the high leaf concentration of salts (Cl^- and/or Na^+) and B^+ in RW irrigated trees, which reduced leaf gas exchange and consequently promoted canopy warming. The amount of

water applied (FI or RDI) also interfered in the behaviour of both physiological parameters. Thus, RDI trees showed a decrease in stomatal aperture and an increase in T_c.

In this study, correlations of T_c, T_c-T_a and CWSI *vs* g_s were tested under several thermal data average intervals, VPD and PAR conditions. Results suggested that g_s values were more closely related to the preceding 60 min averaged T_c values than with timepoint T_c data. This fact indicated that g_s integrated previous values of T_c, which in turn depended on meteorological and plant hydric conditions. In relation with VPD it was observed that within a range between 0 and 2 kPa, T_c increased simultaneously with VPD while g_s remained stable, leading to a decrease in the strength of thermal indices-g_s correlations. A more direct relationship between thermal indices and g_s was found at PAR equal to 1200 µmol m^{-2} s^{-1}, than when PAR exceeded 1200 µmol m^{-2} s^{-1}. This is due to a PAR value higher than 1200 µmol m^{-2} s^{-1} a reduction of g_s was observed, indicating that it is the saturating PAR of grapefruit trees.

In relation to the different phenological stages studied, in FS the best indicators were the normalised thermal indices (T_c-T_a and CWSI), and in FG the absolute values of T_c. This fact indicated that T_c was less sensitive to changes in g_s values when climatic conditions were less demanding as occurred in FS stage. Nevertheless, in FG, the increase in the atmospheric demand gave rise to the T_c was more influenced by plant intrinsic factors (greater or lesser stomatal opening) than by T_a.

Conclusions

The robustness of the different thermal indices (T_c, T_c-T_a and CWSI) for estimating g_s was the highest at 60 min thermal average data, at VPD between 0 and 3.5 kPa, and at PAR 1200 µmol m^{-2} s^{-1}. At these conditions, trees under RDI and those irrigated with RW showed the best thermal indices-g_s correlations. CWSI and T_c-T_a estimated g_s better during FS and T_c during FG, independently of quality of the irrigation source and the amount of water applied. Overall, thermal-based indices have demonstrated to be a useful tool to estimate g_s in grapefruit trees irrigated with different strategies and water sources, and to be able to precise irrigation management.

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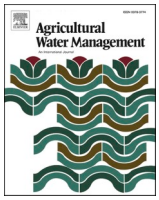

Estimation of stomatal conductance by infra-red thermometry in citrus trees cultivated under regulated deficit irrigation and reclaimed water

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ABSTRACT

The aim of this study was to evaluate the robustness of infra-red thermometry to estimate stomatal conductance (g_s) in grapefruit trees. For this purpose, the ability of several thermal indices to determine g_s was tested for different: (i) irrigation strategies: full irrigation (FI) and regulated deficit irrigation (RDI), (ii) water sources: transfer water (TW) -optimal for agricultural uses- and saline reclaimed water (RW) and (iii) phenological stages: flowering-sprouting (FS) and fruit growth (FG). During two growing seasons, measurements of g_s and canopy temperature (T_c) were taken in 14-year-old Star Ruby grapefruit trees (*Citrus paradisi* Macf.,). Air temperature (T_a) was also recorded and then the derived thermal indices: T_c-T_a, and CWSI (crop water stress index) were calculated. Subsequently, thermal-g_s correlations were established at different averaged thermal data intervals, and VPD (vapour pressure deficit) and PAR (photosynthetically active radiation) conditions. The results indicated that both, water quality and deficit irrigation, affected negatively g_s and T_c during FG. In addition, the highest r^2 values in thermal indices-g_s correlations were found when i) the thermal data was averaged 60 min before the g_s measurement, ii) g_s was measured over a wide range of VPD (between 0 and 3.5 kPa), and iii) PAR was 1200 μmol m^{-2} s^{-1}. The ability of the thermal indices to estimate g_s improved under RDI and RW conditions, when compared to the control treatment (TW-FI). Moreover, the thermal indices that more accurately estimated g_s were T_c-T_a and CWSI in FS ($P < 0.001$), and the absolute values of T_c in FG ($P < 0.001$). Overall, thermal indices obtained by infra-red sensors could be a useful tool to estimate g_s in grapefruit trees. However, irrigation treatment, water quality, tree phenological stage and environmental conditions (VPD and PAR) must be considered when interpreting thermal indices-g_s correlations.

1. Introduction

Citrus is one of the main fruit tree species cultivated, accounting for 16% (138 Mt) of the total fruit production worldwide (FAOSTAT, 2020). Spain is the most important Mediterranean citrus-growing country with a production of 6.0 Mt, being the main exporter of grapefruit in Europe, with a 48% of total European exports of this crop (FAO, 2021). Citrus production is highly dependent on the availability of water resources, which in turn is affected by the ongoing climate change scenario (IPCC, 2018). Water shortages negatively affect citrus yield and fruit quality (Capra et al., 2011; Romero-Trigueros et al., 2020), leading to important economic losses. To avoid these undesirable effects, an optimal use of irrigation water is needed. In this sense, the use of irrigation strategies such as regulated deficit irrigation (RDI) contributes to increase water productivity (Puig-Sirera et al., 2021), with null or low affection of yield and fruit quality (Coyago-cruz et al., 2019). Similarly, irrigation with non-conventional water sources such as reclaimed water (RW) is a good option because it mitigates the problem of water shortage (Li et al., 2021). However, both techniques require proper irrigation management because RDI and RW can negatively affect both the soil and the crop (Romero-Trigueros et al., 2017).

Irrigation scheduling based on plant water status indicators could also be of interest since they allow to match crop water needs with its supply (Fernández, 2017). Previously, stem water potential (Ψ_{stem}), stomatal conductance (g_s) and trunk diameter variations were used as plant water stress indicators and/or for irrigation scheduling purposes (Hernandez-Santana et al., 2019; Intrigliolo et al., 2011; Mirás-Avalos et al., 2016). However, their direct measurements are punctual, laborious and time consuming (Grant et al., 2006) and do not allow real-time irrigation scheduling, unless sensors that measure continuously, as

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dendrometers (Mirás-Avalos et al., 2016) or leaf turgor pressure sensors, are used. In this sense, canopy temperature (T_c), could be easily, remotely, and rapidly measured using infra-red thermometry sensors (IRTs) (Cifre et al., 2005; Jones, 2004), as opposed to g_s and Ψ_{stem}. In addition, IRTs allow to study a large population of leaves (Bellvert et al., 2016) and their measurements can also be extrapolated from the leaf to the field scale (González-Dugo et al., 2014). These characteristics make T_c and its derived thermal indices, the difference between canopy and air temperature (T_c-T_a) and the crop water stress index (CWSI), interesting for irrigation scheduling purposes. In several studies (Baluja et al., 2012; Ben-Gal et al., 2009; Pagay and Kidman, 2019), correlations between thermal indices and traditional indicators of plant water status (g_s and Ψ_{stem}) were detected. In grapefruit trees under saline and water stresses, strong correlations were found between several thermal indices (T_c, T_c-T_a and CWSI) measured by IRTs and Ψ_{stem} (Romero-Trigueros et al., 2019). However, grapefruit has recently been defined as an isohydric species (Romero-Trigueros et al., 2021), since it shows a rapid stomatal closure in response to water stress conditions. Thus, in this crop, g_s is a more reliable indicator of plant water status than Ψ_{stem}. However, there is a lack of knowledge about the usefulness of the different thermal indices (T_c, T_c-T_a and CWSI) for estimating g_s, in grapefruit trees under saline and water stresses. In addition, little is known in reference to what time of day, corresponding to a given VPD (vapour pressure deficit) and PAR (photosynthetically active radiation) conditions, it is best to correlate IRT readings and g_s measurements.

Therefore, the main objective of this study was to estimate g_s using IRTs in grapefruit trees cultivated under different irrigation strategies: full irrigation (FI) and regulated deficit irrigation (RDI) and combined with two water sources: transfer water (TW) and saline reclaimed water (RW). The specific objectives were: (i) to assess the seasonal trend of g_s and T_c; (ii) to evaluate the influence of various irrigation strategies, water sources, and crop phenological stages in thermal indices *vs* g_s correlations and (iii) to determine which thermal index correlated best with g_s. In addition, the ability of different thermal indices to determine g_s was tested at different averaged thermal data intervals (60 min and timepoint) and VPD (VPD_{global} and $VPD_{\leq 2}$) and PAR (PAR_{1200} -equal to saturating irradiance- and $PAR_{>1200}$ -over saturating irradiance-) conditions. In this sense, the working hypothesis were that IRTs can be useful for estimating g_s and that correlations between different thermal indices and g_s will change depending on treatment, crop phenological stage, different thermal data intervals, and environmental conditions.

2. Materials and methods

2.1. Plant material and experimental conditions

The experiment was carried out in a 0.5 ha of the commercial orchard "Lo Montero" located in Molina de Segura, Spain (38°07′18″N, 1°13′15″W) during two growing seasons (2019 and 2020). The experimental plot was cultivated with adult (14 years old, planted in 2005) Star Ruby grapefruit trees (*Citrus paradisi* Macf.,) grafted onto *Citrus macrophylla* L. rootstocks planted at 6 m x 4 m. The irrigation system consisted of a single lateral drip line with four pressure-compensated emitters of 4 L h^{-1} per tree, located at 0.8 m from the axis of the trunk. Irrigation was scheduled based on crop evapotranspiration (ET_c) accumulated during the previous week. ET_c was calculated multiplying the reference evapotranspiration (ET_0, Allen et al., 1998) and specific crop coefficients (K_c) for grapefruit trees (Pedrero et al., 2015).

All the trees were fertilised equally (215, 110 and 150 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively) through the drip irrigation system. Weeds were eradicated by applying pest control methods commonly used by farmers.

2.2. Water sources and irrigation strategies

The experimental plot was irrigated with two water sources: transfer water (TW) and saline reclaimed water (RW). The TW was pumped from the Tajo-Segura canal and the RW from the tertiary wastewater treatment plant (WWTP) in Molina de Segura. The TW was considered optimal water for agricultural use due to its low values of electrical conductivity (EC), close to 1 dS·m^{-1} (average value for 2019 and 2020: 0.91 ± 0.10 dS·m^{-1}). The RW was characterised by having a high salt content and an EC value close to 4 dS·m^{-1}. The RW was automatically mixed in the irrigation control-head with TW to reduce its EC value ≈ 3.5 dS·m^{-1} (average value for 2019 and 2020: 3.19 ± 0.52 dS·m^{-1}) in order to avoid significant yield losses (Maas, 1993). The high level of salinity in RW (Table 1) was the result of a high concentration of Cl^-, Na^+ and B^+, which was approximately 7 times higher than in TW. Moreover, the concentration of K^+ and NO_3^- was also high in this saline water source.

Two irrigation strategies were applied for each water source: full irrigation (FI) and regulated deficit irrigation (RDI). In the FI treatments (TW-FI and RW-FI), trees were irrigated at 100% of ET_c during the experimental period. However, the trees cultivated under the RDI regime (TW-RDI and RW-RDI) were also irrigated at 100% ET_c except in phase II of fruit growth (from 1st July to 15th September), when they were irrigated at 50% of ET_c. These irrigation strategies (FI and RDI) and water sources (TW and RW) have been applied since 2007, allowing the assessment of long-term effects of these practices on grapefruit tree performance. Thus, the combined treatments evaluated were: TW-FI, RW-FI, TW-RDI and RW-RDI. TW-FI was considered the control treatment in this study. The annual amounts of water applied in the FI treatments (TW-FI and RW-FI) were 5125.2 and 4978.4 m^3 ha^{-1} in 2019 and 2020, respectively. However, in the RDI treatments (TW-RDI and RW-RDI) they were 3930.1 and 3941.1 m^3 ha^{-1} in 2019 and 2020, respectively.

The data obtained were separated into two distinct experimental periods, referred to P_1 and P_2. P_1 was just before the RDI period and comprised from 1st April to 30th June 2019 and 2020 (DOY 91–181 and 92–182, respectively). This period coincided mainly with the phenological stages of flowering-sprouting (FS) and with low evaporative demand months. P_2 referred to the RDI period and included the time interval between 1st July and 15th September 2019 and 2020 (DOY 182–258 and 183–259, respectively). In these months, the evaporative demand was high and the main phenological stage was fruit growth (FG).

The experiment was laid out in a completely randomised design with four replications per treatment. In each replicate, the trees were distributed in 3 rows (12 trees in total) and measurements were taken on 1 tree of the central row. The rest of the trees were considered as border trees.

Table 1
Physical and chemical properties of transfer water (TW) and saline reclaimed water (RW).

Property	Units	TW	RW
EC	dS m^{-1}	0.91 ± 0.10	3.19 ± 0.52
pH	–	7.86 ± 0.13	8.77 ± 0.22
Ca^{++}	mg L^{-1}	99.42 ± 6.88	170.82 ± 7.76
Mg^{++}	mg L^{-1}	46.47 ± 6.01	117.33 ± 11.56
K^+	mg L^{-1}	6.00 ± 1.98	40.42 ± 1.90
Na^+	mg L^{-1}	81.09 ± 26.34	550.28 ± 43.81
B^+	mg L^{-1}	0.12 ± 0.04	0.82 ± 0.06
Cl^-	mg L^{-1}	113.43 ± 32.88	749.83 ± 61.46
NO_3^-	mg L^{-1}	2.29 ± 0.61	10.65 ± 3.91
PO_4^{--}	mg L^{-1}	1.19 ± 0.32	1.65 ± 0.72
SO_4^-	mg L^{-1}	266.97 ± 38.67	795.63 ± 120.82

Values are averages ± SE (standard error) of 24 individual samples collected during the experimental period (2019 and 2020). EC: electrical conductivity (dSm^{-1}); RW: reclaimed water; TW: transfer water.

2.3. Measurements

Meteorological variables such as rainfall, air temperature (T_a), relative humidity (RH) ET_0 and solar radiation (R_s), were recorded in real time in a weather station located at the experimental site. The VPD was calculated every hour using the measured T_a and RH data.

The gas exchange parameters, g_s and net photosynthesis (A), were measured with a portable photosynthesis system (LI-6400, LICOR, Lincoln, NE, USA), equipped with a broad leaf chamber (6 cm^2). Inside the measuring chamber, the ambient CO_2 concentration and airflow rate were almost constant, being 400 μmol mol^{-1} and 300 μmol s^{-1}, respectively. Measurements were taken on mature leaves located on the sunny side of the trees, on three leaves per tree and four trees per treatment (one per replicate).

During the experimental period, T_c was continuously monitored using one infra-red sensor per tree (Apogee instruments, Utah, USA, model SIF-111). The sensors were mounted on galvanised metal poles and installed approximately 0.5 m above the tree crown. The sensors had a field of view (FOV) of 22° and were connected to data loggers (model CR1000, Campbell Scientific Ltd., Shepshed, UK), which read values every 5 min and recorded average values every 15 min. T_c was measured on four trees per treatment (one per replicate). The average values of T_c were normalised by calculating the T_c-T_a and CWSI indices as described in Romero-Trigueros et al. (2019).

2.3.1. Study of seasonal trend of g_s and T_c

Stomatal conductance measurements were carried out periodically between 08.00 and 10.00 GMT, depending on the season of the year, and at saturating light intensity (1200 μmol m^{-2} s^{-1}) and at ambient air temperature and relative humidity. Canopy temperature was

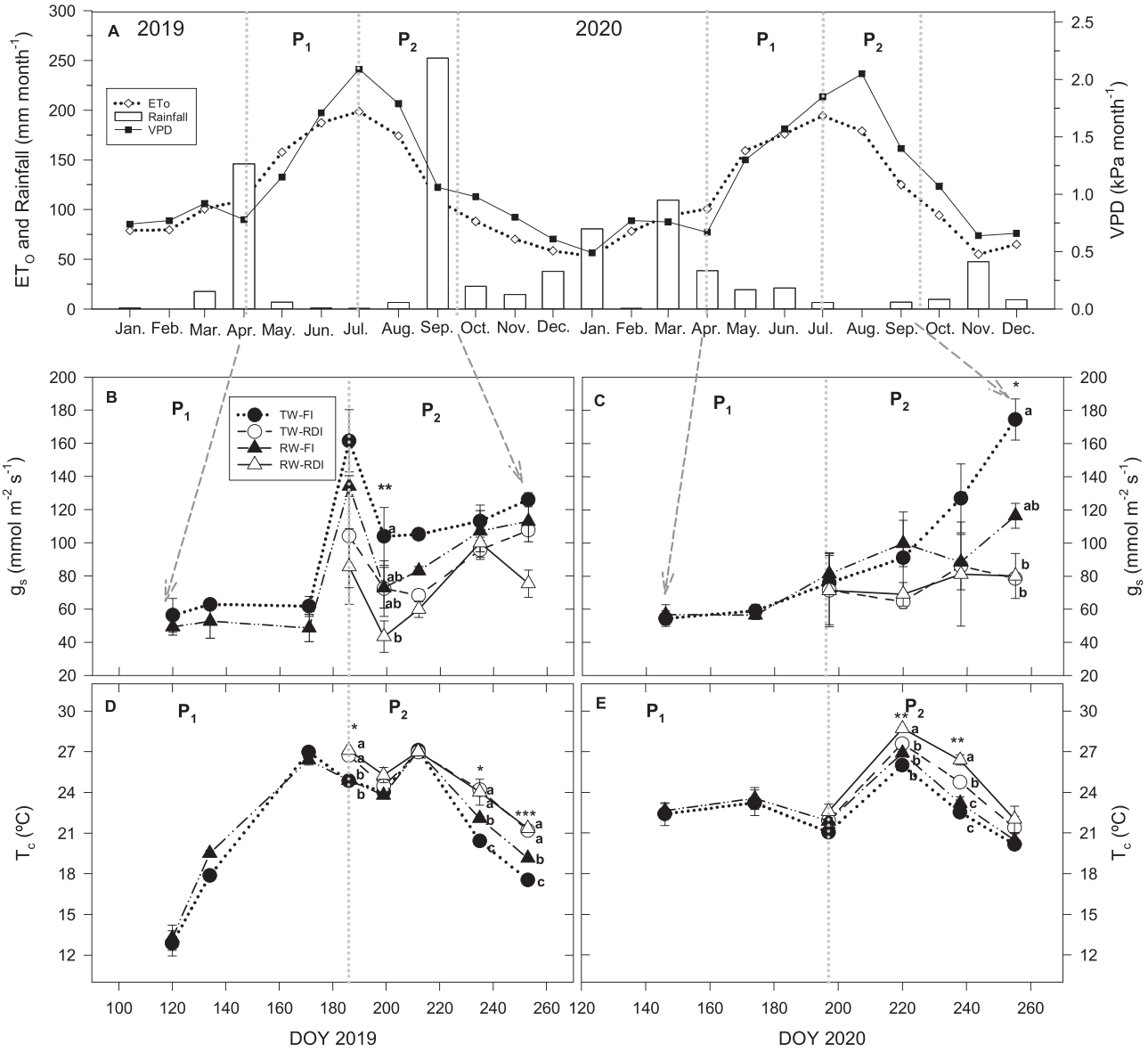

Fig. 1. Monthly data of vapour pressure deficit (VPD), reference evapotranspiration (ET_0), and rainfall of 2019 and 2020 (A). Seasonal trend of stomatal conductance (g_s) (values at 9:00 h solar time) and canopy temperature (T_c) (at the time of the g_s measurement) of grapefruit trees during P_1 (just before RDI period) and P_2 (coinciding with RDI period) of 2019 (B and D) and 2020 (C and E), respectively, for the different treatments studied: transfer water- full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water - full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). Asterisks: *, ** and *** indicate statistically significant differences among treatments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. In each measurement day, different letters indicate significant differences among treatments according to the Tukey's test ($P < 0.05$). T_c and g_s values are means of 4 replications. Vertical bars on data points are ± standard error (SE).

continuously monitored using infra-red sensors and data were collected at the time to g_s measurement to assess the seasonal trend.

2.3.2. Study of time interval of thermal data, and VPD and PAR conditions

2.3.2.1. Time interval of thermal data. The time interval over which the thermal data are averaged could vary the significance of the relationships between the thermal indices (T_c, T_c-T_a and CWSI) and g_s. For this reason, two-time intervals of thermal data were studied: timepoint and preceding 60 min average data (60 min, henceforth). The timepoint data referred to the T_c value at the time of the g_s measurement. The 60 min included all the values of T_c and T_a recorded during the hour prior to the time of measurement of g_s. Therefore, the derived thermal indices (T_c-T_a and CWSI) were calculated in two different ways: i) considering the thermal timepoint data, and ii) using the preceding 60 min average data of T_c and T_a. In a previous test, the preceding 15 and 30 min average thermal data were also evaluated. However, these data are not included here because of strength of the relationship between thermal indexes (T_c, T_c-T_a and CWSI) and punctual stomatal conductance (g_s) was lower at these thermal timeframes (preceding 15 and 30 min average thermal data) than at timepoint and preceding 60 min average thermal data. Then, the thermal indices (T_c, T_c-T_a and CWSI) were correlated with the punctual g_s measurements at the two-time intervals cited above and taking into account the two experimental periods separately: P_1 and P_2 of both years, 2019 and 2020.

2.3.2.2. VPD conditions. To find out the degree of influence of VPD on g_s and T_c measurements of our experiment (Fig. 2), VPD and these two physiological parameters (T_c and g_s) were measured in control treatment trees (TW-FI) on different days and times, during the two growing seasons (2019 and 2020). Measurements of g_s were carried out at saturating light intensity (1200 μmol m^{-2} s^{-1}) and at ambient air temperature and relative humidity. VPD values were collected at the same time that g_s measurements were performed.

In addition, a diurnal course of VPD and g_s was carried out in P_2 of 2019 in all the treatments studied. Measurements of g_s were carried out hourly at saturating light intensity (1200 μmol m^{-2} s^{-1}) and at ambient air temperature and relative humidity. VPD values were collected at the same time that g_s measurements were performed. Subsequently, the hourly values of g_s and VPD were correlated (see Fig. 1S Supplementary Material) across treatments. Then, the thermal indices were correlated with g_s values at two different VPD levels: $VPD_{\leq 2}$, which comprised g_s and thermal data measured at VPD equal or below 2 kPa, and VPD_{global}, which included all g_s and thermal data, regardless of VPD at the time of measurement. In our study, VPD_{global} ranged from 0 to 3.5 kPa.

2.3.2.3. PAR conditions. PAR could have an effect on the thermal indices-g_s correlations. To test this influence, PAR and the two physiological parameters (g_s and T_c) were measured in the control treatment (TW-FI) in different days and hours during two growing seasons (2019 and 2020) (Fig. 3).

In addition, a PAR-photosynthesis curve was made for each experimental period (P_1 and P_2) in the control treatment (TW-FI) (Fig. 2S Supplementary Material). Then, the thermal indices were correlated with g_s values at two different PAR levels: PAR_{1200} (equal to saturating irradiance) and $PAR_{>1200}$ (over saturating irradiance). $PAR_{>1200}$ (over saturating irradiance) was a non-fixed value and ranged between 1300 and 1600 μmol m^{-2} s^{-1}, depending on the environmental PAR condition at the time of g_s measurement.

2.4. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's multiple test ($P < 0.05$) were performed to assess differences between treatments. Before ANOVA analysis, the assumptions of normality and homoscedasticity were checked. Relationships between T_c and VPD, g_s and PAR, and thermal indices (T_c, T_c-T_a and CWSI) and g_s were fitted to linear regressions. Their coefficients of determination (r^2) are shown in the Figs. 2B, 3, 4 and 5. Then, the coefficients of correlations (r) were calculated and used to assess the strength of these relationships. The relationships between g_s and VPD were fitted to quadratic regressions. The statistical analysis was performed with IBM SPSS (version 25, SPSS Inc., Armonk, NY, USA 2002).

3. Results

3.1. Meteorological conditions and seasonal trend of g_s and T_c

Meteorological data for 2019 and 2020 are shown in Fig. 1A. The total annual rainfall and ET_0 were 508.20 mm and 1413.23 mm, respectively, in 2019, and 351.01 mm and 1374.47 mm, respectively, in 2020. The maximum rainfall was recorded in September 2019, accounting for the 49.64% of the annual rainfall. In P_1, before the RDI period, weather conditions were milder than in P_2, with an average value of VPD and ET_0 of around 1 kPa day^{-1} and 5 mm day^{-1} in both years studied (Fig. 1A). During P_1 of 2019 and 2020, g_s and T_c values were quite similar in TW-FI and RW-FI trees, regardless of water source (Fig. 1B, C and D, E, respectively).

In P_2, coinciding with the RDI period and the summer months, the average values of VPD and ET_0 were around 3 kPa day^{-1} and 8 mm day^{-1}, respectively in 2019 and 2020 (Fig. 1A). In contrast to P_1, in this period, significant differences in g_s (Fig. 1B, C) and in T_c (Fig. 1D,

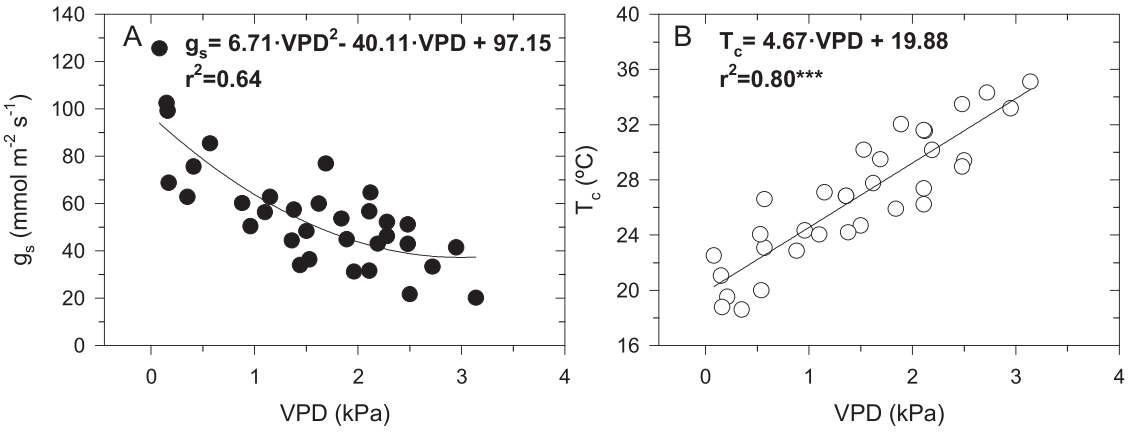

Fig. 2. Relationship between: (A) stomatal conductance (g_s) and (B) canopy temperature (T_c) with VPD in P_1 (just before RDI period) and P_2 (coinciding with RDI period) of 2019 and 2020. Each point is the mean of 4 replications of the control treatment: transfer water - full irrigation (TW-FI). The level of significance of the relationship is shown as: *** ($P < 0.001$).

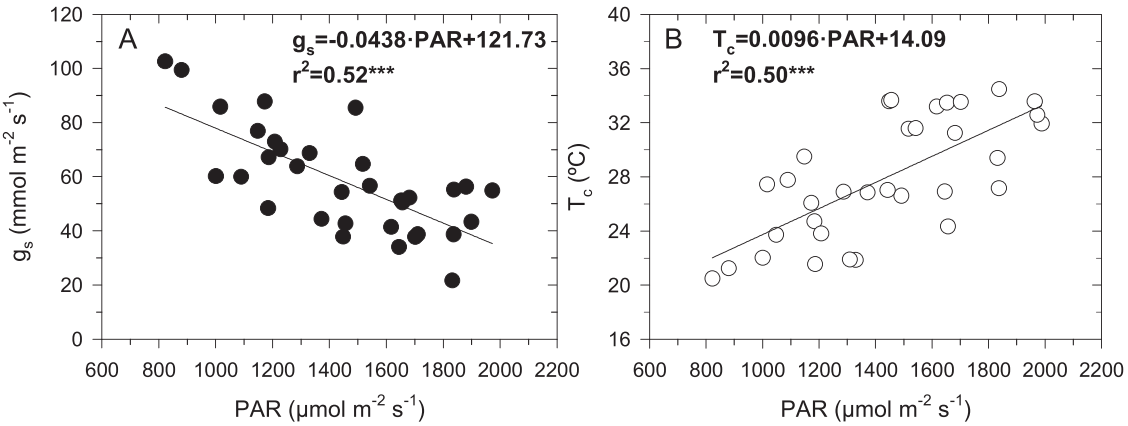

Fig. 3. Relationship between: (A) stomatal conductance (g_s) and (B) canopy temperature (T_c) with photosynthetically active radiation (PAR) in P_1 (just before RDI period) and P_2 (coinciding with RDI period) of 2019 and 2020. Each point is the mean of 4 replications of the control treatment: transfer water-full irrigation (TW-FI). The level of significance of the relationships is shown as: *** ($P < 0.001$).

E) were found among treatments. This was especially evident in the case of T_c, with significant differences between treatments on several days (Fig. 1 D, E). The highest values of g_s and the lowest values of T_c were observed in the FI treatments (RW-FI and TW-FI), and the opposite behaviour was observed in the RDI treatments (RW-RDI and TW-RDI) (Fig. 1B, C, D and E, respectively). In September 2019, coinciding with the rainiest month, g_s and T_c values were quite similar in the different irrigation treatments studied (Fig. 1B, D).

The global seasonal average of T_c was higher in P_2 (23.96 °C) than in P_1 (19.48 °C), as expected, coinciding with the months of high evaporative demand. The same trend was also observed for g_s, with higher values in P_2 (96.58 mmol m^{-2} s^{-1}) than in P_1 (55.19 mmol m^{-2} s^{-1}).

3.2. Relationships between punctual measurements of gs and thermal indices at different time intervals, VPD and PAR conditions

The assessment of the relationships between the three thermal indices (T_c, T_c-T_a and CWSI) and g_s at different thermal data intervals, VPD and PAR conditions are described in the Section 3.2.1., 3.2.2. and 3.2.3.

3.2.1. Thermal data time intervals: timepoint and preceding 60 min

Correlations between the different thermal indices and g_s considering the two thermal time intervals are shown in Table 2. An overall increase in the r^2 value and on the significance of the correlations in the 60 min data was observed, respect to timepoint data.

Considering the different treatments and only this average data interval (60 min), there was no clear trend in P_1, with higher r^2 values, in some cases, for the trees irrigated with RW and, in other cases, for the trees irrigated with TW (Table 2). However, in the second period (P_2), the RDI trees irrigated with RW showed the highest r^2 values, except for the T_c-T_a *vs* g_s correlation, where the RW-FI treatment showed the highest degree of correlation.

3.2.2. VPD conditions

In our experiment, T_c and g_s were strongly influenced by VPD conditions, with r^2 value of 0.64 and 0.80 ($P < 0.001$) for the correlations between T_c and g_s with VPD, respectively (Fig. 2).

Thus, VPD was a key variable for appropriately estimating g_s from thermal indices. Besides, it is important to know the VPD value above which the influence on g_s and T_c is greatest.

We observed that g_s increased while VPD values ranged from 0 to 1.8 kPa (Fig. 1S, see Section 2.3.2). However, when VPD values were higher than 1.8 kPa, a turning point occurred, from which g_s values began to decrease. This behaviour was more accentuated from 2.0 kPa, threshold value of VPD that we used to study the influence of VPD on the correlations between g_s and thermal indices.

When thermal indices-g_s correlations were evaluated at the two different VPD levels: $VPD_{\leq 2}$ and VPD_{global}. In general, higher r^2 values were observed for the VPD_{global} conditions than for $VPD_{\leq 2}$, in the three correlations studied (T_c *vs* g_s, T_c-T_a *vs* g_s, CWSI *vs* g_s) (Table 3). Only in some exceptions, the $VPD_{\leq 2}$ condition had r^2 values higher than VPD_{global}. Considering only VPD_{global} data, all correlations between thermal indices *and* g_s were significant.

Taking into account only VPD_{global} data, concerning water sources, in P_2, the highest r^2 values were observed for trees irrigated with RW (Table 3). Nevertheless, in P_1, no clear trend was observed. Regarding the amount of water applied (FI and RDI treatments), there was no clear pattern in P_2 (Table 3). For instance, in the T_c *vs.* g_s correlation, the RW-RDI treatment showed a r^2 higher than that obtained for RW-FI. In other cases, the r^2 values were similar in the FI and RDI treatments. For example, in the CWSI *vs.* g_s correlation, the RW-RDI and RW-FI treatments showed r^2 values of 0.55 and 0.58, respectively. Overall, both RW and RDI treatments increased the magnitude of r^2 in the thermal indices *vs* g_s correlations with respect to the control treatment (TW-FI).

3.2.3. PAR conditions

In our study, T_c and g_s of trees were strongly influenced by PAR levels throughout the vegetative cycles of 2019 and 2020, with r^2 value up to 0.5 ($P < 0.001$) in both cases (Fig. 3). In such a way that, the higher PAR corresponded with a higher g_s (Fig. 3A) and T_c values (Fig. 3B).

In the PAR- photosynthesis curves (Fig. 2S Supplementary Material and see Section 2.3.2), when PAR was higher than 1200 µmol m^{-2} s^{-1}, a photo-inhibition was observed, leading to a reduction of g_s values. For this reason, the threshold value of 1200 µmol m^{-2} s^{-1} was considered key to study the influence of PAR in the estimation of g_s by thermal data.

When thermal indices-g_s correlations were plotted considering the two different PAR levels: PAR_{1200} and $PAR_{>1200}$, the results pointed to a higher r^2 at PAR_{1200} than at $PAR_{>1200}$ in all correlations studied (Table 4). In the T_c *vs* g_s correlations, this was especially evident for RW-FI treatment, which showed an r^2 of 0.52 ($P < 0.01$) at PAR_{1200} and an r^2 of 0.25 (no significant) at $PAR_{>1200}$. In CWSI *vs* g_s correlations, all treatments showed a higher r^2 for PAR_{1200}. The same trend was observed in T_c-T_a *vs* g_s correlations, with high r^2 values in almost all treatments when PAR_{1200} was considered.

As for the periods, in P_1, and at PAR_{1200}, there was no definite trend since the highest r^2 were shown, in some cases, by the RW and, in other cases, by the TW (Table 4). On the contrary, in P_2, the use of RW and/or RDI generally improved the significance of the correlations respect to the control treatment (TW-FI).

Thus, the highest r^2 values of the correlations between thermal indices (T_c, T_c-T_a and CWSI) and g_s were shown at 60 min averaged

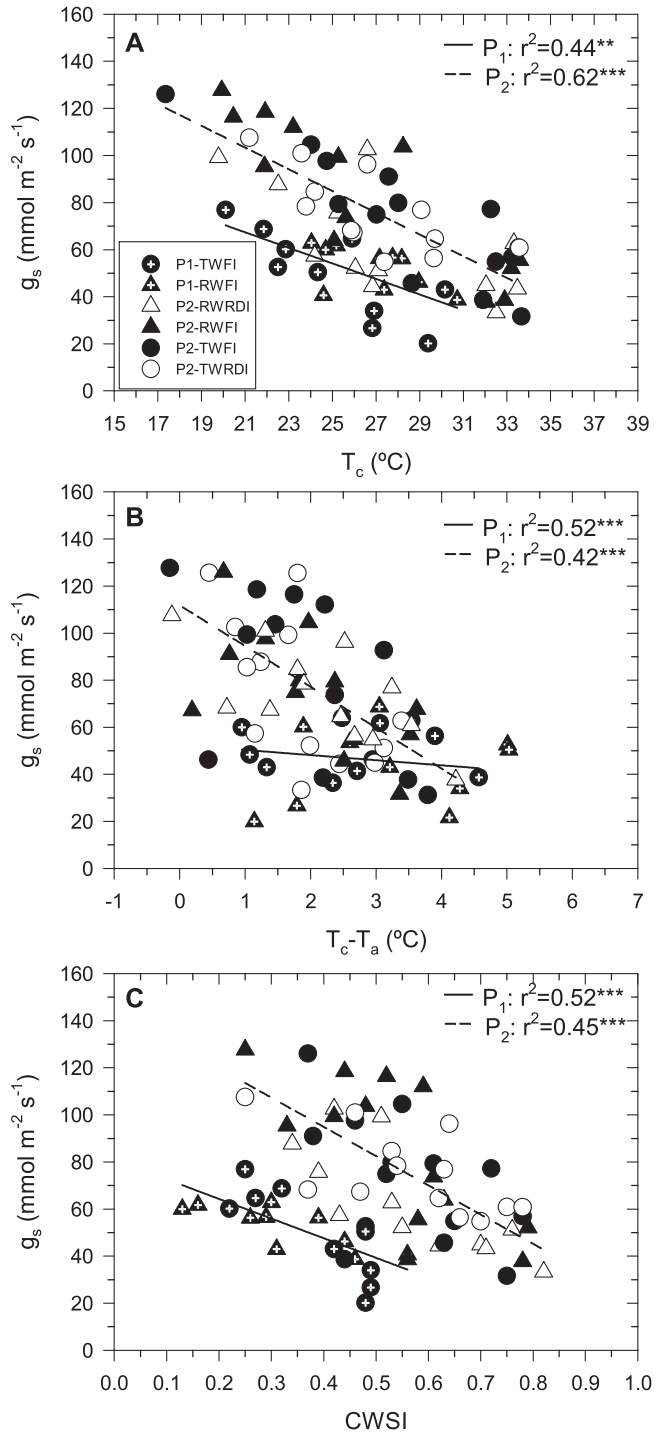

Fig. 4. Relationship between stomatal conductance (g_s) and (A) canopy temperature (T_c), and its derived thermal indices: (B) canopy to air temperature difference (T_c-T_a) and (C) crop water stress index (CWSI) for the different treatments studied: transfer water - full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water-full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). The regressions were adjusted into two distinct experimental periods: P_1 (just before RDI period) and P_2 (coinciding with RDI period) of both years, 2019 and 2020. Each point is the mean of 4 replications. The level of significance of the relationships is shown as: ** ($P < 0.01$), and *** ($P < 0.001$), respectively. The equations for T_c *vs* g_s: P_1 ($g_s = -3.30 \cdot T_c + 136.67$) and P_2 ($g_s = -4.62 \cdot T_c + 200.35$); the equations for T_c-T_a *vs* g_s: P_1 ($g_s = -8.21 \cdot T_c\text{-}T_a + 72.73$) and P_2 ($g_s = -17.36 \cdot T_c\text{-}T_a + 111.84$); the equations for CWSI *vs* g_s: P_1 ($g_s = -83.42 \cdot CWSI + 80.99$) P_2 ($g_s = -124.24 \cdot CWSI + 144.56$).

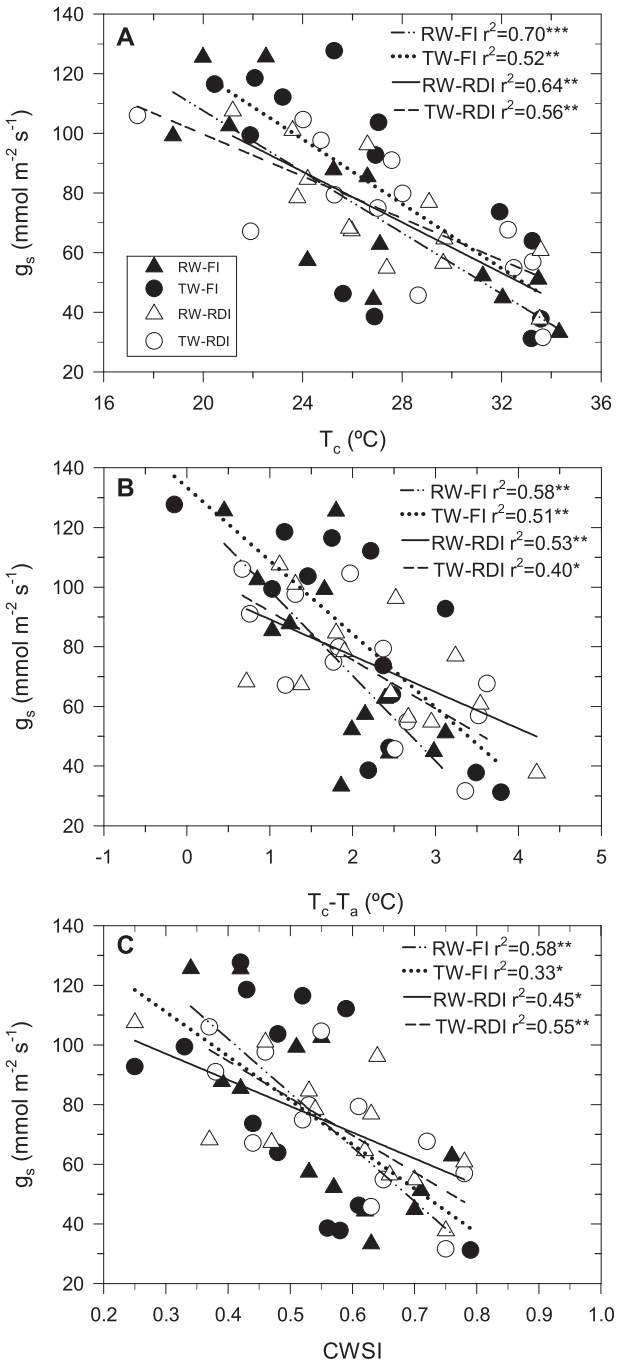

Fig. 5. Relationship between stomatal conductance (g_s) and (A) canopy temperature (T_c), and their derived thermal indices (B) canopy to air temperature difference (T_c-T_a) and (C) crop water stress index (CWSI) for the different treatments studied: transfer water - full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water - full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). The data was from P_2, which coincided with the RDI periods of both years, 2019 and 2020. Each point is the mean of 4 replications. The level of significance of the relationships is shown as: * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$), respectively. The equations for T_c *vs* g_s were: RW-FI: $g_s = -5.12 \cdot T_c + 209.98$, TW-FI: $g_s = -5.41 \cdot T_c + 227.91$, RW-RDI $g_s = -4.25 \cdot T_c + 189.06$ and TW-RDI $g_s = -3.52 \cdot T_c + 170.2$. The equations for T_c-T_a *vs* g_s were: RW-FI: $g_s = -28.61 \cdot T_c\text{-}T_a + 127.5$, TW-FI: $g_s = -24.61 \cdot T_c\text{-}T_a + 133.48$, RW-RDI: $g_s = -12.21 \cdot T_c\text{-}T_a + 101.4$ and TW-RDI: $g_s = -16.3 \cdot T_c\text{-}T_a + 108.15$. The equations for CWSI *vs* g_s were: RW-FI: $g_s = -181.69 \cdot CWSI + 174.70$, TW-FI: $g_s = -148.08 \cdot CWSI + 155.48$, RW-RDI: $g_s = 87.87 \cdot CWSI + 123.42$ and TW-RDI: $g_s = -124.52 \cdot CWSI + 144.40$.

Table 2
Coefficients of determination (r^2) of the relationships between T_c *vs* g_s, T_c-T_a *vs* g_s and CWSI *vs* g_s for the different treatments studied: transfer water - full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water - full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). The regressions were adjusted into two experimental periods: P_1 (just before the RDI period) and P_2 (coinciding with RDI period) of both years, 2019 and 2020, and considering two thermal time intervals: timepoint and preceding 60 min average data. The level of significance is shown as: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) and ns 'not significant'.

		P_1^1		P_2^2			
		TW-FI[3]	RW-FI	TW-FI[3]	TW-RDI	RW-FI	RW-RDI
T_c *vs* g_s	60 min	0.41 *	0.62 **	0.64 ***	0.64 ***	0.52 **	0.70 ***
	timepoint	0.30 ns	0.44 *	0.65 ***	0.59 **	0.47 **	0.74 ***
T_c-T_a *vs* g_s	60 min	0.69 **	0.56 *	0.40 *	0.55 **	0.60 **	0.40 *
	timepoint	0.54 *	0.45 *	0.33 *	0.57 **	0.40 *	0.28 ns
CWSI *vs* g_s	60 min	0.61 **	0.65 **	0.35 *	0,41 *	0.55 **	0.58 **
	timepoint	0.66 **	0.59 **	0.32 *	0.27 ns	0.48 **	0.39 *

[1]60 min and timepoint n = 10; [2]60 min and timepoint n = 13; [3]TW-FI was considered in this study as the control treatment.

Table 3
Coefficients of determination (r^2) of the relationships between T_c *vs* g_s, T_c-T_a *vs* g_s and CWSI *vs* g_s for the different treatments studied: transfer water - full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water - full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). The regressions were adjusted into two experimental periods: P_1 (just before the RDI period) and P_2 (coinciding with RDI period) of both years, 2019 and 2020, considering two VPD conditions: VPD_{global} and $VPD_{\leq 2}$. The level of significance is shown as: * ($P < 0.05$), ** ($P < 0.01$) *** ($P < 0.001$) and ns 'not significant'.

		P_1^1		P_2^2			
		TW-FI[3]	RW-FI	TW-FI[3]	TW-RDI	RW-FI	RW-RDI
T_c *vs* g_s	VPD_{global}	0.41 *	0.62 **	0.64 **	0.64 **	0.52 **	0.70 ***
	$VPD_{\leq 2}$	0.27 ns	0.48 ns	0.46 *	0.61 *	0.53 *	0.62 *
T_c-T_a *vs* g_s	VPD_{global}	0.69 **	0.56 *	0.40 *	0.55 **	0.60 **	0.40 *
	$VPD_{\leq 2}$	0.58 *	0.77 **	0.49 *	0.71 **	0.37 ns	0.13 ns
CWSI *vs* g_s	VPD_{global}	0.60 **	0.65 **	0.35 *	0.41 *	0.55 **	0.58 **
	$VPD_{\leq 2}$	0.52 ns	0.53 ns	0.44 ns	0.71 **	0.31 ns	0.37 ns

[1]VPD_{global} n = 10 and $VPD_{\leq 2}$ n = 7; [2]VPD_{global} n = 13 and $VPD_{\leq 2}$ n = 9; [3]TW-FI was considered in this study as the control treatment.

Table 4
Coefficients of determination (r^2) of the relationships between T_c *vs* g_s, T_c-T_a *vs* g_s and CWSI *vs* g_s for the different treatments studied: transfer water - full irrigation (TW-FI), transfer water - regulated deficit irrigation (TW-RDI), reclaimed water - full irrigation (RW-FI) and reclaimed water - regulated deficit irrigation (RW-RDI). The regressions were adjusted into two experimental periods: P_1 (just before the RDI period) and P_2 (coinciding with RDI period) of both years, 2019 and 2020, considering two PAR conditions: PAR_{1200} and $PAR_{>1200}$. The level of significance is shown as: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) and ns 'not significant'.

		P_1^1		P_2^2			
		TW-FI[3]	RW-FI	TW-FI[3]	TW-RDI	RW-FI	RW-RDI
T_c *vs* g_s	PAR_{1200}	0.41 *	0.62 **	0.64 **	0.64 **	0.52 **	0.70 ***
	$PAR_{>1200}$	0.48 *	0.12 ns	0.41 *	0.34 *	0.25 ns	0.43 *
T_c-T_a *vs* g_s	PAR_{1200}	0.69 **	0.56 *	0.40 *	0.55 **	0.40 *	0.40 *
	$PAR_{>1200}$	028 ns	0.34 ns	0.40 *	0.21 ns	0.44 *	0.35 *
CWSI *vs* g_s	PAR_{1200}	0.61 **	0.65 **	0.35 *	0.41 *	0.55 **	0.58 **
	$PAR_{>1200}$	0.34 ns	0.31 ns	0.28 ns	0.28 ns	0.26 ns	0.38 *

[1]PAR_{1200} and $PAR_{>1200}$ n = 10; [2] PAR_{1200} and $PAR_{>1200}$ n = 13; [3]TW-FI was considered in this study as the control treatment.

thermal data and at VPD_{global} and PAR_{1200}, so that these three conditions were used in the relationships described in the next section.

3.3. Relationships between g_s and thermal indices in different experimental periods and irrigation treatments

On the one hand, the three correlations (T_c *vs* g_s, T_c-T_a *vs* g_s and CWSI *vs* g_s) were, first, globally adjusted, without taking into account treatments and periods. The r^2 values obtained were significant (data not shown), however, the level of significance of these correlations increased when the different experimental periods were considered (Fig. 4). The r^2 values ranged from 0.42 to 0.62, depending on the thermal index and the period (Fig. 4A, B and C). In general, all correlations showed a high significance level ($P < 0.01$ and $P < 0.001$).

In P_1, coinciding with the phenological stage of FS, the highest r^2 values (r^2 =0.52, $P < 0.001$) were observed for the normalised thermal indices (T_c-T_a and CWSI). Unlike what was observed in P_1, in P_2, which coincided with the phenological stage of FG, the absolute values of T_c showed a higher r^2 value (r^2 =0.62, $P < 0.001$) than the normalised thermal indices *vs* g_s.

On the other hand, to study the influence of irrigation treatments and water quality in thermal indices *vs* g_s correlations, we considered the four treatments of P_2: RW-FI, RW-RDI, TW-FI and TW-RDI (Fig. 5). In all the correlations studied, RW-FI trees showed slightly greater r^2 values than TW-FI trees, and the same trend was observed for the RDI treatments (RW-RDI and TW-RDI) (Fig. 5). As an example, in the case of T_c *vs* g_s correlation, the value of r^2 for TW-FI was 0.52 and for TW-RDI, RW-FI and RW-RDI were 0.56, 0.70 and 0.64, respectively. In addition, the absolute value of T_c was the best index in P_2 (Fig. 4) since, the r^2 values were higher for the T_c *vs.* g_s regressions (Fig. 5A) than for those considering the two normalised thermal indices (Fig. 5B, C).

4. Discussion

4.1. Influence of water source and irrigation strategy on g_s and T_c dynamics in different crop phenological stages

In this work, prior to the evaluation of estimation of g_s using IRT sensors, we assessed whether the use of RW or deficit irrigation at different crop phenological stages can influence the dynamics of T_c and g_s of grapefruit trees. The measurements of g_s and T_c which were carried out in the different treatments during FS (P_1) and FG (P_2) phenological stages of 2019 and 2020 suggested that when the weather conditions were mild (P_1) water quality had no effect in g_s and T_c seasonal dynamics, in agreement with what was observed previously by Romero--Trigueros et al. (2014) in the same crop. However, when the weather conditions were more demanding, stomatal aperture was greater in all trees, compared to P_1. This favouring of stomatal aperture could be aimed at increasing CO_2 uptake and subsequently photosynthetic capacity and production of photo-assimilates, which are necessary for fruit development. A recent study in lime trees (Mira-García et al., 2021) also observed an increase in g_s coinciding with fruit development stage. In addition, warming of the canopy was also observed in P_2, as a consequence, among other factors, of an increase in T_a during the summer months, as T_c is mainly driven by environmental factors such as T_a or RH (Pineda et al., 2021). In contrast, both RW and RDI influenced stomatal regulation of grapefruit trees in P_2 and, therefore, they affected canopy warming dynamics. As for water quality, TW-FI trees showed a higher stomatal conductance than RW-FI trees, due to the high leaf concentration of salts (Cl^- and/or Na^+) and B^+ in trees irrigated with RW (Romero-Trigueros et al., 2017), which reduce leaf gas exchange (Pedrero et al., 2015). The amount of water applied (FI or RDI) also interfered in the behaviour of both parameters. RDI trees showed a decrease in stomatal aperture, in agreement with what has been observed by other authors (Anjum, 2008; González-Dugo et al., 2019; Melgar et al., 2008) in *Citrus*. Regardless of treatment, T_c showed the opposite response to g_s, as expected, in such a way that a high stomatal aperture induced a reduction in canopy temperature and *vice versa* (Costa et al., 2012).

4.2. Relationship between thermal indices and g_s at different time intervals, VPD and PAR conditions

In this study, one of the main objectives was to estimate g_s by different thermal indices: T_c, T_c-T_a and CWSI. However, the relationship between g_s and thermal indices could be influenced by the time interval in which temperature data are averaged and the VPD and PAR conditions when g_s was measured. Therefore, as a novelty of this work, correlations of T_c, T_c-T_a and CWSI *vs* g_s were tested under several thermal data average intervals (60 min and timepoint), VPD ($VPD_{\leq 2}$ and VPD_{global}) and PAR (PAR_{1200} and $PAR_{>1200}$) conditions.

The results suggested that g_s values were more closely related to the preceding 60 min averaged T_c values than with timepoint T_c data, suggesting that g_s integrated previous values of canopy temperature, which in turn depends on meteorological (Grant et al., 2016) and plant hydric conditions (Romero-Trigueros et al., 2019). In a previous unpublished study, the authors also evaluated other thermal data average intervals (15 and 30 min), but their results are not shown here since the r^2 values obtained with these intervals were lower than those obtained at 60 min

Moreover, meteorological conditions such as VPD also influence the values of g_s (Diaz-Espejo et al., 2007) and T_c (Mahan et al., 2016). Here, VPD was correlated with T_c so that an increase in VPD promoted a linear increase in canopy heating. Nevertheless, unlike T_c, the influence of VPD on g_s changed depending on VPD conditions: at low VPD conditions ($VPD_{\leq 2}$), stomatal conductance was less sensitive to changes of VPD, as also pointed out by Yáñez et al. (2021), than T_c. This is, as VPD increase, within a range between 0 and 2 kPa, T_c increased simultaneously with VPD while g_s remained stable, leading to a decrease in the strength of thermal indices-g_s correlations. An additional meteorological condition which influenced the dynamic of the stomata was PAR, as observed in our results and in a recent study also performed on grapefruit trees (Romero-Trigueros et al., 2019). Another recent study performed on lime trees reported that a 53% increase in PAR led to a 46% reduction in the accumulated daily g_s value (Mira-García et al., 2020). In our work, both physiological parameters, g_s and T_c, were influenced by PAR, with high r^2 values between them. In the case of T_c, an increase in PAR caused a direct canopy warming. However, in the case of g_s, the clear reduction in g_s values when PAR exceeded 1200 μmol m^{-2} s^{-1} indicated that this value is the saturating PAR in grapefruit trees. This justified why when thermal indices-g_s correlations were assessed at two PAR levels (PAR_{1200} and $PAR_{>1200}$), a more direct relationship between T_c and g_s was found at PAR equal to 1200 μmol m^{-2} s^{-1}.

4.3. The best thermal index for estimating g_s

A significant r^2 value between thermal indices-g_s was found in several species, such as almond trees (González-Dugo et al., 2012), vineyards (Baluja et al., 2012), and spinach (Masseroni et al., 2017). Here, in CWSI *vs* g_s correlation, r^2 values were slightly lower than those observed in other studies performed on *Firmiana platanifolia* (Yu et al., 2015), sunflowers planted in pots (Nielsen and Anderson, 1989), and vines cultivated in soil (Belfiore et al., 2019). The same was observed for the T_c-T_a *vs* g_s correlation, where the r^2 value was slightly lower here than for a 'Clementina de Nules' commercial orchard (Ballester et al., 2013). This could be because in those studies T_c-T_a and CWSI were calculated from thermal image data and not from IRT sensors data (like in our study), and normally the thermal images are taken on the same part of the tree where g_s is measured (Leinonen et al., 2006). Here, the measurements of g_s were performed in the lower part of the canopy, while our IRT sensors took measurements in the upper part of the canopy. This fact indicates that the accuracy of our model could be improved by carrying out future trials where both physiological variables (T_c and g_s) are measured in the same area of the tree. Moreover, the correlations of our work were assessed at two different phenological stages: P_1 and P_2 coinciding with the phenological stages of flowering-sprouting and fruit growth, respectively. The fact that most suitable indicators in P_1 were the normalised thermal indices (T_c-T_a and CWSI), and in P_2 the absolute values of T_c, indicated that T_c was less sensitive to changes in g_s values when climatic conditions were less demanding. In P_1, the crop temperature depended mainly on external climatic conditions rather than plant water status. On the contrary, in P_2, the increase in the atmospheric demand gave rise to the canopy temperature was more influenced by plant intrinsic factors (greater or lesser stomatal opening) than air temperature, according to Shoa et al. (2021). Thus, thermal measurements needed less normalisation in P_2, in line with another study (Meron et al., 2013) in which climatic conditions were evaluated. Besides, we also evaluated the influence of treatments on the thermal indices *vs* g_s correlations. For all treatments studied (RW-FI, RW-RDI, TW-FI and TW-RDI), T_c was the best thermal indicator for more accurately estimating g_s in P_2.

Stomatal conductance has already used for irrigation scheduling in other crops (Hernandez-Santana et al., 2019) and is the plant water status indicator more reliable for grapefruit (Romero-Trigueros et al., 2021). For this, in future trials, after improving the accuracy of the correlations, it would be advisable to validate the use of g_s (estimated by thermoradiometers) to schedule irrigation in grapefruit trees. That is, to define the optimal g_s values in the different phenological stages and irrigation strategies, and then to translate these g_s values into threshold values of thermal indices, for an efficient irrigation management.

In summary, the ability of thermal indices obtained by thermometry to estimate g_s in grapefruit trees under RW and RDI was evaluated here. The results can be summarised in the following statements: i) the value of g_s at a given moment integrated the T_c of the previous 60 min; ii) the estimation of stomatal conductance by IRT improved when g_s and T_c

values were collected during a wide range of VPD (should not be limited to VPD<2 kPa) and when PAR did not exceed 1200 μmol m^{-2} s^{-1}; iii) the absolute value of T_c was the most suitable thermal indicator in all treatments during fruit growth stage, and iv) the ability of T_c to estimate g_s improved when RW and RDI strategies were used, compared with TW-FI.

5. Conclusion

The robustness of the different thermal indices (T_c, T_c-T_a and CWSI) for estimating stomatal conductance of grapefruit trees was tested for the first time on several thermal data intervals and environmental conditions. The results suggested that thermal indices-g_s correlations had the highest degree of correlation when preceding 60 min thermal average data, VPD_{global} and PAR_{1200} were considered. In these time intervals and environmental conditions, trees under deficit irrigation and those irrigated with reclaimed water showed an increase in the thermal indices-g_s r^2 values. We also found the most suitable thermal indicator in each of the different phenological stages and irrigation treatments studied. Normalised thermal indices were the most robust during flowering-sprouting (P_1), and the absolute values of T_c during fruit growth phenological stage (P_2), independently of quality of the irrigation source and the amount of water applied.

Nevertheless, the maximum variance explanation achieved was around 70% due to, among other factors, the g_s measurements were performed in the middle part of the tree canopy, while IRT sensors were placed above the canopy. Thus, in future research the precision of these relationships should be improved by determining the proper part of the tree where g_s and T_c should be measured. Then, it would be interesting to test the capacity of the model for scheduling irrigation and/or estimation of the threshold of g_s or T_c at which irrigation should be triggered or stopped.

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CRediT authorship contribution statement

Ana Belén Mira-García: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Cristina Romero-Trigueros:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision. **José María Bayona Gambín:** Methodology. **María del Puerto Sánchez-Iglesias:** Methodology. **Pedro Antonio Nortes Tortosa:** Conceptualization. **Emilio Nicolás Nicolás:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2022.108057.

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CHAPTER V: GENERAL CONCLUSIONS

The main conclusions derived from this thesis, which is aimed to integrate novel multidisciplinary approaches to the sustainability of precision irrigation on citrus crops (grapefruit and lime) grown under Mediterranean conditions, were:

Lime water requirements

- Under Mediterranean conditions and without water limitation, evapotranspiration (ET) values of two-year-old lime trees (*Citrus latifolia* Tan. cv. Bearss) grown in 45 L pots ranged through the crop cycle from 0.25 to 2.56 L plant^{-1} and day^{-1}.

- For day-length periods, when changes in tree biomass were negligible, pot weight served for ET-water balance validation in lime trees.

- A prototype of a simple and robust scalable drainage-weighing lysimeter, with real-time monitoring of irrigation and soil water balance parameters, provided a practical and cost-effective way to measure ET in lime trees, with potential use in other crops.

Soil and plant water status indicators threshold values for precise irrigation management

- Automated irrigation protocol, based on the soil maximum allowed depletion (MAD) concept, ensured an optimal soil and lime trees water status.

- In the dried soil curve, the breaking point that connected the rapid and the slower volumetric soil water content (θ_v) decrease was indicative of the beginning of severe water stress in lime trees, allowing a precise irrigation management.

- Midday canopy-to-air temperature difference (T_c-T_a) threshold values proposed for triggering drip irrigation in lime trees grown in semi-arid conditions were 2.4 and 0.8 ◦C for open-field and shaded conditions, respectively, when irrigation water availability was sufficient to fulfil plant water requirements. Under deficit irrigation conditions, the proposed values were 2.9 and 1.3 ◦C for moderate water deficits (stem water potential, Ψ_{stem} = -1.5 MPa) and 3.4 or 1.8 ◦C, for severe water deficits (Ψ_{stem} = -2 MPa), for open-field and shaded lime trees, respectively.

Effect of cropping system on plant and soil water status indicators

- Soil and plant water status indicators values were affected by diurnal and seasonal changes and by cropping system (shading and open-field).

- Cropping system mainly affected the leaf gas exchange, with the highest stomatal conductance (g_s) values in lime trees cultivated in shaded conditions.

- Shading favoured plant cooling, as indicated by the more negative T_c-T_a values in this cropping system with respect to open-field conditions.

- Under netting a better soil-plant water status was promoted, delaying plant water stress appearance and resulting in an interesting cropping technique to cope with climate change in semi-arid areas.

- Cropping system must be considered to propose T_c-T_a threshold values for lime tree drip irrigation management.

Discrete *vs.* continuous plant water status indicators

- In grapefruit trees, correlations between canopy temperature (T_c), T_c-T_a and crop water stress index (CWSI) with g_s had the highest degree of correlation when: (i) thermal data was averaged 60 min before g_s measurements, (ii) at vapor pressure deficit (VPD) between 0 and 3.5 kPa, and (iii) at photosynthetically active radiation (PAR) 1200 μmol m^{-2} s^{-1}.

- Normalised thermal indices (T_c-T_a and CWSI) were the most robust to estimate g_s in grapefruit trees during flowering-sprouting, and the absolute T_c values during fruit growth phenological stage, regardless of quality of irrigation source and the amount of water applied.

- Thermal-based indices served as an alternative to other classical discrete physiological measurements, as g_s and Ψ_{stem}, for remotely and real-time monitoring lime and grapefruit water status.

Effects of water source and irrigation strategy on plant water status

- Grapefruit trees under regulated deficit irrigation and those irrigated with reclaimed water showed the best thermal indices-g_s correlations.

- Regulated deficit irrigation and irrigated with reclaimed water promoted stomatal closure and consequently an increase in canopy warming in grapefruit trees.

CHAPTER VI: REFERENCES

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ANNEX I: IMPACT FACTOR

Article I

Mira-García, A.B., Vera, J., Conejero, W., Conesa, M.R., Ruiz-Sánchez, M.C., 2021. Evapotranspiration in young lime trees with automated irrigation. Scientia Horticulturae 288, 110396. DOI: 10.1016/j.scienta.2021.110396

Impact factor: 4.342, **Quartile:** Q1, **Category:** Horticulture and **Rank:** 4/37

Article II

Mira-García, A.B., Conejero, W., Vera, J., Ruiz-Sánchez, M.C., 2020. Leaf water relations in lime trees grown under shade netting and open-air. Plants 9(4), 510. DOI: 10.3390/plants9040510

Impact factor: 3.935, **Quartile:** Q1, **Category:** Plant Sciences and **Rank:** 47/235

Article III

Mira-García, A.B., Conejero, W., Vera, J., Ruiz-Sánchez, M.C., 2022. Water status and thermal response of lime trees to irrigation and shade screen. Agricultural Water Management 272, 107843. DOI: 10.1016/j.agwat.2022.107843

Impact factor: 6.611, **Quartile:** Q1, **Category:** Agronomy and **Rank:** 5/90

Article IV

Mira-García, A.B., Romero-Trigueros, C., Bayona-Gambín, J.M., Sánchez-Iglesias, M.P., Nortes-Tortosa, P.A., Nicolás-Nicolás, E., 2023. Estimation of stomatal conductance by infrared thermometry in citrus trees cultivated under regulated deficit irrigation and reclaimed water. Agricultural Water Management 276, 108057 DOI: 10.1016/j.agwat.2022.108057

Impact factor: 6.611, **Quartile:** Q1, **Category:** Agronomy and **Rank:** 5/90