Dipartimento di Agricoltura, Alimentazione e Ambiente
Di3A

UNIVERSITÀ DEGLI STUDI DI CATANIA

PhD

in

AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCE

XXXIII Cycle

Deficit irrigation of orange trees: perspectives and main effects

Daniela Saitta

Advisor:
Prof.ssa Simona Consoli

Coordinator:
Prof. Cherubino Maria Leonardi

Ph. D. attended during 2017/2020

Abstract

The sustainable management of water in agriculture through the use of deficit irrigation strategies (DI) represents a valid opportunity to maximize irrigation efficiency, in particular for the Mediterranean areas characterized by a tending decrease in water availability and a gradual but continuous increase in temperatures and crops evapotranspiration demand (ETc).

The objective of the PhD consisted in the evaluation of the applicability of DI strategies on a mature orange grove (Citrus sinensis (L.) Osbeck cv Tarocco Sciara) in order to monitor the effects on its physiological and biochemical response, on the yield and on quality, compared to the full irrigation regime (FI). The study also made it possible to identify the adaptation characteristics of the orange grove under study to deficit irrigation strategies, which were administered for about a decade in the experimental area.

The study also proposed to compare and evaluate the accuracy of monitoring evapotranspiration (ET_c) *and transpiration (T) flows through the adoption of measurement techniques [Eddy Covariance (EC) and heat pulse velocity of the lymph flow (HPV)] and approaches based on estimation methods (FAO-56 model).*

The results of the doctoral work show how important it is to carry out accurate monitoring of the physiological and biochemical parameters of crops subjected to deficient irrigation regimes, in order to promptly identify the onset of non-reversible stress conditions.

Based on the results obtained, it can be deduced that deficit irrigation strategies (DI) can be successfully applied to high value cultivation systems, such as the orange grove under study (Citrus sinensis (L.) Osbeck cv Tarocco Sciara), without the deficit imposed compromises the qualitative and quantitative yield characteristics, with the advantage of reaching considerable levels of water savings (up to 53% in cases of greater water reduction), with consequent increases in water use efficiency (WUE) compared to well-irrigated theses. To this end, the work conducted over the three-year period of the doctorate suggests the possibility of adopting deficit irrigation strategies for orange groves (Citrus sinensis (L.) Osbeck cv Tarocco Sciara), from the earliest stages of transplanting in the field, in order to to allow conditions of progressive and gradual adaptation.

Sommario

La gestione sostenibile dell'acqua in agricoltura attraverso l'uso di strategie di irrigazione deficitaria (DI) rappresenta una valida opportunità per massimizzare l'efficienza irrigua, in particolare per le aree del Mediterraneo caratterizzate da una tendente diminuzione della disponibilità idrica e da un graduale ma continuo aumento delle temperature e della richiesta evapotraspirativa delle colture (ET_c).

L'obiettivo del dottorato di ricerca è consistito nella valutazione dell'applicabilità di strategie di DI su un aranceto maturo (Citrus sinensis (L.) Osbeck cv Tarocco Sciara) al fine di monitorare gli effetti sulla sua risposta fisiologica e biochimica, sulla resa e sulla qualità, rispetto al regime di piena irrigazione (FI). Lo studio ha, inoltre, consentito di identificare le caratteristiche di adattamento dell'aranceto in studio alle strategie di DI, che sono state

somministrate per circa un decennio presso l'area sperimentale.

Lo studio si è altresì proposto di confrontare e valutare l'accuratezza del monitoraggio dei flussi di evapotraspirazione (ET_c) e di traspirazione (T) attraverso l'adozione di tecniche di misura [Eddy Covariance (EC) e velocità dell'impulso di calore del flusso di linfa (HPV)] e di approcci basati su metodi di stima (modello FAO-56).I risultati del lavoro di dottorato evidenziano quanto sia importante effettuare un monitoraggio accurato dei parametri fisiologici e biochimici delle colture sottoposte a regimi irrigui deficitari, al fine di identificare prontamente l'insorgenza di condizioni di stress non reversibili.

In base ai risultati ottenuti si può dedurre che le strategie di irrigazione deficitaria (DI) possono essere applicate con successo a sistemi colturali di alto valore, come l'aranceto in studio (Citrus sinensis (L.) Osbeck cv Tarocco Sciara), senza che il deficit imposto comprometta le caratteristiche di resa qualitativa e quantitativa, con il vantaggio di raggiungere livelli ragguardevoli di risparmio idrico (fino al 53% nei casi di maggiore decurtazione idrica), con conseguente aumenti dell'efficienza d'uso dell'acqua (WUE) rispetto alle tesi ben irrigate.

A tal fine il lavoro condotto nell'arco del triennio di dottorato suggerisce la possibilità di adottare strategie di irrigazione deficitaria per gli aranceti (Citrus sinensis (L.) Osbeck cv Tarocco Sciara), fin dalle prime fasi di trapianto in piano campo, al fine di consentirne condizione di progressivo e graduale adattamento.

PART I
Introduction
1. Deficit irrigation perspectives

About 70% of Earth's surface is interested by the presence of water (Küppers et al., 2014; Siddique and Bramley 2014), but only about 2.5% is freshwater (Gleick and Palaniappan 2010). The majority of water is trapped in glaciers, permanent snow, or aquifers (Farihi et al., 2013; Sivakumar 2011). Water shortages threaten many parts of the world, with nearly 800 million people lack access to safe drinking water and 2.5 billion have no proper sanitation (Schiermeier 2014).

The situation may get worse in coming decades, as world's population is expected to increase by 30 % by 2050 (Godfray et al., 2010) coupled with forecasted climate change (de Wit and Stankiewicz 2006). Agriculture is the largest freshwater user on the planet, consuming more than two thirds of total withdrawals (Gan et al., 2013). In many parts of the world, irrigation water has been over-exploited and over-used (Chai et al., 2014), and freshwater shortage is becoming critical in the arid and semiarid areas of the world (Forouzani and Karami 2011). Therefore, deficit irrigation strategies such as such as regulated deficient irrigation (RDI) and partial root zone drying (PRD) play an important role in ensuring good water conservation in agriculture (Chai et al., 2014).

Deficit irrigation was born in 1980 as an economic strategy, aimed at maximizing the economic benefit of the farmer. It was then subsequently declined in various concepts linked to the analysis of the physiological, productive and qualitative characteristics linked to its effects on the different crops.

Studies (Silveira et al., 2020; Gasque et al., 2016; Ruiz-Sánchez et al., 2010) have shown that the use of these strategies have no negative consequences on fruit yield or quality, with significant water savings and improvements in water use efficiency. Therefore, where water resources are limited these strategies represent an excellent prospect for the future.

1.1 Morphology and phenological phases of citrus fruits

Citrus is the most widely cultivated fruit crop worldwide, with an annual production of 135.7 million metric tons during 2013 (FAOSTAT, Database). The most commercially important *Citrus* species are oranges (*C. sinensis* L. Osbeck) and tangerines (*C. unshiu* Marc., *C. nobilis* Lour., *C. deliciosa* Ten., *C. reticulate* Blanco and their hybrids) with more than 80 %, followed by lemons (*C. limon* L. Burm. f.), limes (*C. aurantifolia* Christm. Swing.) and grapefruits (*C. paradise* Macf.) in almost equal proportions (Zaher-Ara et al., 2016) Citrus fruits are evergreen plants probably originating in China or India, they belong to the rutaceae family. They require a climate with limited temperature ranges and in particular in the summer they require relatively high temperatures and abundant water availability. Orange, lemon and mandarin take on considerable importance in the southern regions of Italy and in Sicily. They are trees of medium or limited development or shrubs, with alternate leathery leaves, elongated in shape with a bright green upper surface. The fruit is a berry called hesperidium with a round shape and variable size, the pulp is divided into wedges (8-12 cloves).

Sweet orange (Citrus sinensis) is the most common citrus species in Italy. The plant reaches a good development of 8-

12 m, the leaves are whole, the fruits tend to be spherical or oval with bright orange peel. The orange cultivars are distinguished by the color of the pulp in blond and pigmented. Among the blonde cultivars there are: Washington Navel, Valencia Late, Ovale Calabrese, Bionda Comune

Among the cultivars with pigmented pulp (due to the presence of anthocyanins in the pulp) we find: Moro, Tarocco and Sanguinello. The Tarocco, which is the crop of our interest, compared to the others, has larger fruits and smoother and thinner peel and medium ripeness (late December - January).

In our environmental conditions, citrus fruits perform three vegetations: one in spring and two in summer, the first is important for production purposes, as it is the most abundant and develops a greater number of flowers than the tree's fruit setting capacity; the summer vegetation allows to accumulate the reserves used in the following season. The growth of citrus fruits has a trend that can be divided into three phases (**Figure 1**):

- phase 1: from flowering to the physiological drop in June;
- phase 2: from the drop in June to the pre-veraison of the fruits;
- phase 3: from veraison to fruit ripening and senescence.

During phase 1, which has a variable duration depending on the species from 40 to 50 days, the growth of the fruit is rapid, the increase in size occurs by cell division. Phase 2 has a variable duration between 2-3 and 5-6 months, depending on the variety, and growth occurs by cellular distension. Phase 3, called ripening, begins when the skin changes color with an internal transformation of the fruit that makes it juicier, with an increase in soluble solids and a constant decrease in the content of organic acids. The growth and development of citrus fruits is affected by climatic variables. Climatic conditions directly induce certain physical qualities (Cronje et al., 2013), alter phenophase times (Webb et al., 2007) and also determine the level of fruit maturity (Porras et al., 2014).

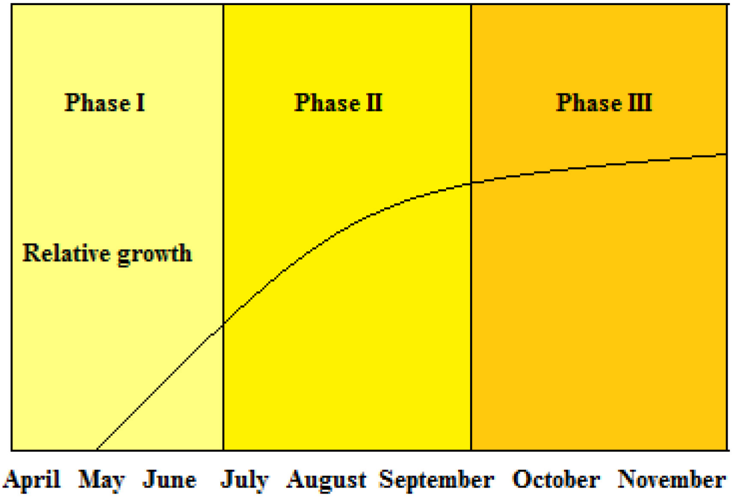

Figure 1. Fruit growth stages (from Quaderno Agrumicoltura 2009 - modified).

1.2 Regulated Deficit Irrigation

One of the most promising approaches to increase the use of water resources in agriculture is the Regulated Deficit Irrigation (RDI). The RDI consists of reducing water supplies during certain stages of crop development, when yield and fruit quality might have a low sensitivity to water deficits, and providing normal irrigation during the rest of the season, especially during critical periods or phenological stages with a higher sensitivity to water deficits (Chalmers et al., 1986; Mitchell et al., 1984). The examined literature reports agronomic benefits of RDI obtained for high-density orange groves, olive orchards and pomegranate in terms of fruit quality and size (Peréz-Peréz et al., 2010; García-Tejero et al., 2011; Consoli et al., 2014; Roccuzzo et al, 2014; Selahvarzi et al., 2017; Consoli et al., 2017; Trentacoste et al., 2018). Many studies have shown that this practice reduces water consumption in crops with little or no impact on fruit quality or yield (eg Carr, 2012, Ruiz-Sánchez et al., 2010).

González-Altozano and Castel (2003 a), González-Altozano and Castel (2003 b), González-Altozano and Castel (2000), González-Altozano and Castel (1999) carried out several RDI trials on an experimental citrus orchard "Clementina de Nules" (Clementine citrus Hort ex Tan).

Different levels of water restriction were compared in the main phenological periods of crop development and the effects of water reductions on quality, fruit yield and water use efficiency were evaluated. They concluded that the effects of RDI depend on the phenological period in which the water restriction is applied and on the level of restriction applied. Furthermore, they also showed that a moderate reduction of water during the initial phase of fruit enlargement, after the fall of June which corresponds in **Figure 1** to phase II, did not affect the quality, yield and size of the fruit, but led to good water savings between 8% and 22%.

Studies on the application of RDI demonstrate the advantages of this technique on the reduction of water application during the summer period. This is because citrus fruits have the ability to increase their growth after a period of water deficit and reach their potential size, capacity known as compensatory fruit growth (Gasque et al., 2016).

Ballester et al. (2013) have shown that by carrying out summer RDI treatments on late Navel Lane citrus trees they could prevent the compensatory growth of fruits after return to full-dose irrigation, depending on the duration and degree of severity of the plant's water deficit. This latest study highlights the differences between the cultivars in response to RDI, as well as the need for frequent monitoring of plant water status to avoid excessive reduction in fruit weight which can affect yield.

Regarding the application of long-term RDI strategies, some studies show that this technique can negatively affect the yield (Girona et al., 2005; Intrigliolo et al., 2013; Romero et al., 2004); while other studies report a considerable water saving without any negative influence on the yield and size of the fruits (Hueso e Cuevas, 2010; Johnson et al., 1992).

In studies conducted by María Gasquea et al (2016) regarding the effects of long-term applications of RDI on commercial Navelina citrus trees, it was shown that significant water savings were achieved during the study period (five consecutive growing seasons) between 12% and 27% which determined a slight reduction in the average weight of the fruits in the RDI treatments, in the most restrictive cases, which was balanced with a higher yield.

In any case, to increase the reliability of the strategy, it is always necessary to have a deep knowledge of the physiological growth characteristics of the species under study.

1.3 Partial Root-Zone Drying

Partial rootzone drying (PRD) is a strategy of DI that consists in irrigating only one half of the rootzone in each irrigation event, while the other half is maintained dry. For this, both halves are watered alternately (Dry et al., 1998). This technique was first developed in Australia for vineyards and relies on root-to-leaf signaling induced by a rootzone that is in a drying process (Dry et al., 1996), decreasing stomatal aperture and leaf growth, preventing water loss (Dry et al., 2001; Dodd et al., 2006) with a little effect on photosynthesis, hence increasing transpiration efficiency (Dry et al., 1996). At the same time, the plant maintains an optimal water state because the other half of the root receives water, so that the yield cannot be significantly compromised and the quality can even be improved (Dry et al., 2001). The PRD performance is based on the assumption that photosynthesis and fruit growth are less sensitive to water deficit than transpiration, and besides, water deficit induces the production of chemical signals, like ABA in the root, which reduces leaf expansion and stomatal conductance, while, simultaneously, roots of the watered side of the soil absorb sufficient water to maintain a favorable plant water status (Liu et al., 2006; Zegbe et al., 2006; Ahmadi et al., 2010). As demonstrated in a recent analysis, the advantages of PRD in relation to RDI are highly controversial and also depend on the soil texture, a success or enhanced yield performance with RDI and PRD occurring most likely in deep and finely soils (Adu et al., 2018). Several studies have shown that the water use efficiency (WUE) can be significantly improved with little or no decrease in crop productivity following the use of the PRD technique (Loveys et al., 1997; Kang et al., 2003;

Fernández et al., 2007; Hutton and Loveys, 2011; Ballester et al., 2013; Consoli et al., 2014; Parvizi et al., 2014) thus decreasing canopy vigor and maintain crop yield, if compared with conventional irrigation methods (Intrigliolo and Castel, 2009; Kang et al., 2000; Hutton and Loveys, 2011; Consoli et al., 2014; Consoli et al., 2017). PRD has been successfully adopted in grapevine (De la Hera et al., 2007), pear (Kang et al., 2002), peach (Goldhammer et al., 2001), olive (Fernández et al., 2006) and apple (Talluto et al., 2008) crops. Studies conducted on grapevine (Dry et al., 2000) and citrus fruits (Mary et al., 2019) have shown that in the presence of deficient irrigation, in the specific case PRD, it leads the roots of trees to explore larger volumes of land to obtain access to more water resources that they otherwise would not be able to reach. Kusakabe et al. (2016) tested, in a ripe grapefruit orchard, during two consecutive seasons, three irrigation strategies: drip PRD (two drip lines, alternating irrigation between lines every month), microsprinkler irrigation, and double -line drip irrigation (control). They highlighted that: PRD saved water and had higher WUE crop than control or microsprinkler irrigation; PRD maintained or increased yield compared to microsprinkler irrigation; trees under all irrigation treatments had comparable fruit and juice quality; PRD did not reduce flowering potential or fruit set; there were no significant differences in shoot length among irrigation treatments.

The application of this irrigation strategy is advantageous to avoid the use of large quantities of water that in an arid environment could be lost due to soil evaporation, resulting in poor crop performance and water productivity.

1.4 Soil water content

Soil water content monitoring is important, especially when using deficit irrigation strategies such as RDI and PRD.

The quantity of water contained in the soil can be used to estimate the water state of a crop, and eventually determine the time of intervention. It can be expressed as a percentage of water per unit of mass or volume of soil, or by means of the water potential. The latter represents the force with which water is retained in the interstices, and therefore the work required to remove it from the ground. The lower the water potential value, the greater the force with which the water is retained.

There are numerous instruments capable of detecting the level of humidity, starting with traditional tensiometers, which however are found to be impractical and precise in management and maintenance.

Among the most common methods to measure the water content of a soil sample is the gravimetric method which allows you to calculate the percentage of water on the dry weight (Wd) or on the wet weight (Ww). The sample, after being taken at a certain depth, is weighed to obtain the wet weight and then dried in an oven at 105-110 ° C for 12-48 hours, until it reaches a constant weight (Giardini, 1995).

The percentage is obtained from the formulas:

$$Hd = (Ww - Wd / Wd) * 100 \tag{1}$$

$$Hw = (Ww - Wd / Ww) * 100 \tag{2}$$

If you want to have the percentage by volume (Hv) you need to apply the formula:

$$Hv = Wd * pa \tag{3}$$

where pa is the apparent specific gravity of the soil.

The most commonly used electromagnetic method for estimating soil moisture is (time domain reflectometry: TDR), which is based on the measurement of the dielectric constant (K) of the ground by measuring the propagation speed of an electromagnetic signal (in the 1 MHz - 1 GHz band). The dielectric constant of water is higher (81.5 at 20 ° C) than that of dry soil (2-3). The measured values are therefore proportional to the water content within the soil. Depending on the depth at which you want to make the measurement, you can use different types of probes. The electromagnetic signal is given by a portable instrument suitably connected to the probes, which is able to process the return signal by expressing the water content as a percentage of volume.

2. Micro-irrigation

Compared to other irrigation methods, drip irrigation systems provide the possibility to apply lower volumes of water, more frequently and efficiently. If well designed, these systems make it possible to apply slow, steady and uniform amounts of water and nutrients within the plant’s root zone, while minimizing deep percolation and maintaining high productivity levels (Rallo et al., 2011).

Micro-irrigation is a very spreading irrigation technology, in particular in those areas where water is limited, because it allows to achieve considerable water savings, but also where water is aboundant, but the paradigm of sustainable water resources is paramont. If the drip irrigation system is properly designed, installed, and managed, drip irrigation may help achieve water conservation by reducing evaporation and deep drainage.

To implement the precise localization of the typical deliveries of the method, drip irrigation systems require a dense network of drip lines, generally organized in sectors, which are put into operation one at a time, in cyclic succession. The installation obviously differ in relation to the crop, the cultivation technique, the shape and position of the plots, as well as the business context.

Drip irrigation can be set up at a low initial investment by small-scale farmers with locally available material (for e.g. using buckets or barrels as water reservoir and bamboo or PVC tubes as distribution pipes) (Singh et al., 2000).

2.1 *Surface drip irrigation*

Surface drip irrigation (SDI) uses pipelines in which drippers are included by a co-exstruction process; these can be rigid if they last several years and therefore intended for tree crops, to distribute the water in the soil surface next to the plants o most common flexible for annual crops. The term surface micro-irrigation indicates the particularty of pasing the pipeling in contact with the soil surface (**Figure 2**).

For installing a surface drip irrigation system, it is important to know the climatic conditions and the pedological, hydraulic and agronomic characteristics of the area to be irrigated, as well as the chemical-physical quality of the irrigation water, in order to ensure an excellent functioning. If correctly sized, the surface micro-irrigation allows satisfactory results in terms of uniformity of distribution within the irrigation sector.

Emission uniformity EU has been one of the most frequently used design criteria for micro-irrigation system design. Emission uniformity expresses the emitter flow variation of a microirrigation system affected by hydraulic variation, manufacturer's variation and emitter grouping.

For the micro-irrigation systems, Keller and Karmeli (1975) proposed to evaluate the project uniformity emission as follows:

$$EU = 100\,(1 - 1.27 * CVT/\,n_p^{1/2}) * q_{min}/q_m \qquad (4)$$

where EU = design uniformity coefficient, %; n_p = number of emitters per system; CVT = coefficient of technological variation; q_{min} = flow rate of the emitters operating at the minimum pressure of the sector (l/s or l/h); q_m = flow rate of the distributor operating at the average pressure of the sector (l/s or l/h).

To take into account that the efficiency of irrigation is influenced not only by shortages but also by excess water:

$$EU = 100\,(1 - 1.27 * CVT/\,n_p^{1/2}) * (q_{min}/q_m + q_m/\,q_{max}) \qquad (5)$$

where EU = coefficient of absolute design uniformity%; n_p = number of emitters per system; CVT = coefficient of technological variation; q_{min} = flow rate of the emitters operating at the minimum pressure of the sector (l / s or l / h); q_{max} = flow rate of the emitters operating at the maximum pressure of the sector (l/s or l/h); q_m = flow rate of the distributor operating at the average pressure of the sector (l/s or l/h).

When the irrigation system has already been built, it is necessary to evaluate the EU emission uniformity by measuring, at the operating pressure, the flow rate on a significant number of emitters installed along the dispenser wings.

Emission uniformity (EU,%): the ratio between the average of the lower quartile of the measured flows ($Q_{min1/4}$) and the average of the measured flows (Q_m), in l/h (Keller and Karmeli, 1975).

$$EU = 100 * Q_{min\ 1/4} / Q_m \qquad (6)$$

Percentage of reduction in mean flow rate (Rd,%)

$$R_d = 100* (1- Q_m / Q_t) \qquad (7)$$

Qt is the flow rate of new non-clogged drippers, at the same operating pressure.

In micro-irrigation, the uniformity of water distribution corresponds to the uniformity of flow rate of the emitters in the sector. In sprinkler irrigation, the rainfall diagram of the sprinkler must also be considered.

The plants must be guaranteed the same water volume (V) as:

$$V = q * t \qquad (8)$$

and since for all the emitters of a sector t is constant, q should also be constant.

This irrigation technology allows to administer water near the plants root system, avoiding any losses due to surface runoff or percolation in the deep layers; if well managed the system allows to have a high irrigation efficiency, less development of weeds, the possibility of watering even in the hottest hours of the day, energy saving following low operating pressures, reduction of water losses due to soil evaporation and absence of soil constipation (Provenzano et al., 2005; Incrocci et al., 2004).

Another advantage related to micro-irrigation derives from the possibility of irrigating even in the presence of wind, a not negligible feature in particular climatic conditions.

The main disadvantage is the risk of clogging of the dispensers, for this reason it is always necessary to have an upstream good mesh filter of the size of 50-120 mesh, (depending on the type of dripper used) and a pressure reducer that avoids dangerous changes due to the integrity of the components of the systems (Incrocci, et al., 2004).

Another drawback of this system is the reduced possibility of using water for distribution of pesticides and fungicides or in the anti-freeze and climatic irrigation, since the distribution of water does, generally, not affect the epigean part of the plant.

Figure 2. Drip line in contact with the soil surface

2.2 Sub-surface drip irrigation

Sub-surface drip irrigation (SSDI) consists in the uniformly water distribution at low pressure directly in the root area, keeping the soil surface dry (Martínez-Gimeno et al., 2018). The arrangement of the drip lines is below the soil surface, so the water use efficieny by plant is maximized.

Irrigation is supplied to plants by capillary movement from the bottom. The root-zone air space is not immediately filled by water, in contrast with traditional irrigation where water is supplied directly overhead and water first fills the air space in the soil (Chai et al., 2016).

The installation depth of the drip lines depends on a series of factors such as: the type of crop, the type of soil, the type of water resource used, the climate and the agronomic practices adopted. Many authors have contributed to the evolution of the technique by analyzing both conceptual aspects of the irrigation method and aspects related to the design of distribution networks (Hanson et al., 1997; Van der Gulik, 1999; Burt and Styles, 2007; Lamm and Camp, 2007; Lamm, 2009; Li et al., 2019).

The spread of this irrigation technique was rather slow compared to the surface-drip irrigation technique, because the growers believed that it presented a greater economic risk due to the lack of easily observable indicators that allow to manage the correct functioning and the system performance (Lamm et al., 2012).

Since the 1980s, sub-surface drip irrigation has become the most efficient irrigation system (Robles et al., 2016) and its has been suggested as a promising strategy for a sustainable water management in semiarid regions (Consoli et al., 2014). The plants under subsurface irrigation have been shown to maintain a high leaf turgor potential and a retention of a high symplastic water fraction that help plants to improve morphological strengthening, such as a thicker epidermis and more wax deposits on leaves and cuticle (Chai et al., 2016).

The use of this irrigation system brings numerous advantages including the minimization of water losses by evaporation, drastic reduction of weeds (Provenzano, 2007), an increase in the collection, optimal management of water and fertilizers, low compaction and soil erosion, optimization of field operations, increase in water use efficiency and longer life following protection from ultraviolet rays and thermal excursions (Consoli et al., 2014; Robles et al., 2016; Zhang et al., 2017).

However, the adoption of SSDI has been also associated to some inconveniences such as a high initial cost, potential for rodent damage, salt accumulation between the drip lines and soil surface, and particularly, high potential for emitter clogging (Phene et al., 1986, 1993, 1995).

In relation to the initial investment costs, higher than traditional micro irrigation systems, the use of SSDI can become economically advantageous only if the efficiency of the plant is guaranteed for long periods of the order of 15-20 years (Lamm et al., 2015, 2017).

Research shows also that subsurface irrigation increases crop productivity and production quality. For example, tomato (Solanum lycopersicum L.) seedlings watered through subsurface irrigation dripper system increased both fruit yield and quality compared to the control where water was directly dripped to near the base of the plants (Xu et al. 2011). Subsurface irrigation usually induces osmotic adjustment, increases leaf turgor potential, and consequently enhances photosynthetic activities (Xu et al., 2011).

Robles et al. (2016) carried out studies on lemon trees using surface (SUR) and subsurface (SUB) drip systems for irrigation. The results showed that the SUB system increased the water use efficiency in lemon trees thanks to a water saving of 19% without a negative impact on yield.

Therefore, there is the possibility of saving water in agricultural production and in this sense further research is needed in this direction to help optimize water resources in agriculture.

3. Soil-plant-atmosphere monitoring

The analysis of the complex water interactions that occur in agricultural ecosystems is based on the concept of the continuous soil-plant-atmosphere (SPA) system (Rallo, et al., 2011) which was first theorized by Philip in 1966.

A careful observation of the processes that develop in the Soil - Plant - Atmosphere (SPA) continuum become fundamental for the management of agricultural water, in particular in arid and semi-arid ecosystems (Noy-Meir, 1973), where water is the limiting factor of production and the use of precision irrigation represents a valid management system for this resource (Cammalleri et al., 2013).

The study of this system is very complex, not only for the considerable number of variables that come into play, but also for the phenomena of internal self-regulation that occur between the different components of the system itself.

The movement of water from the ground to the atmosphere is influenced by the hydraulic characteristics of the soil, the morphological and functional characteristics of the plant and the evapotranspiration demand of the atmosphere. The movement of water in the plant is governed by rules similar to those for the flow of electricity, as described by Ohm's law. The water flow in the plant can be described through a network of potentials, of resistances (**Figure 3**). The potential gradient in the soil-plant-atmosphere continuum is the driving force of transport through the plant: the water flow will depart from one point in the system with a high (less negative) water potential to another point with a low (more negative) potential water. Therefore the flow will follow the direction from the ground (Ψs = -0.01 ÷ -0.15 MPa) towards the atmosphere (Ψatm = -50 ÷ -100 MPa) passing through the plant (Mugnai, 2004). It is therefore a passive process exerted through the plant by a negative pressure or suction.

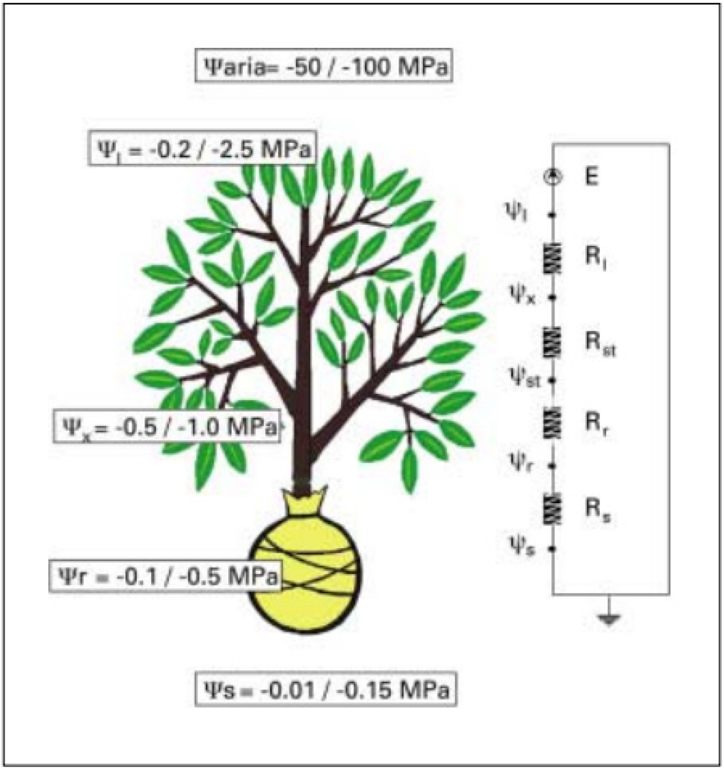

Figure 3. Description of the soil-plant-atmosphere continuum through the analogy with an electrical circuit (from Mugnai 2004).

Ψ_s = soil water potential; Ψ_r = root water potential; Ψ_x = xylematic water potential; Ψ_l = leaf water potential; Ψ_a =

water potential of the atmosphere; R_s = soil resistance; R_r = radical resistance; R_{st} = stems resistance; R_l = leaf resistance; E = external environment.

The monitoring of the water status of the various components of the system can be carried out using different methodologies.

Traditionally, researchers have estimated the soil's moisture content by gravimetric analysis of the extracted samples or by using techniques that measure its dielectric properties (Time Domain Reflectometry). Although these techniques are often accurate, they are point measurements and cannot provide sufficient information on the spatial distribution of soil water content on a large scale. Geophysical methods (Vereecken et al., 2006; Allred et al., 2008; Binley et al., 2015) are potentially effective for monitoring soil-root interactions. In particular, the effect of plant growth, phenological stage, nutrient availability and soil texture on plant root distribution dynamics, combined with the intermittent nature of water inputs, leads to great variability in uptake water from the roots (RWU) (Van Noordwijk et al., 2015).

In this respect, there is a growing demand for near surface observation technologies (eg geophysical methods) to study significant agricultural phenomena in the soil (Bitella et al., 2015). Recent studies (Cassiani et al., 2015; Consoli et al., 2017; Satriani et al., 2015) have shown that these techniques can improve irrigation operations by providing information on optimal irrigation quantities and times. Geophysical methods can also provide indirect, high resolution information on soil moisture distribution and this can prevent excessive water depletion, especially when water deficit conditions are imposed, such as when using the partial drying irrigation technique of the root zone (Romero-Conde et al., 2014). In particular, given the specificity of the PRD, geophysical applications can provide the identification of changes in soil moisture. Electrical resistivity tomography (ERT) (**Figure 4**) is considered to be one of the most effective geophysical methods used in agricultural and environmental studies. This is a minimally invasive method that provides data with high spatial and temporal resolution (Michot et al., 2003; Al Hagrey, 2007). In particular, ERT provides information on the variability of the electrical resistivity (ER) of the subsoil; when considered together with water and solute content, it can help characterize the spatial distribution of water and nutrient uptake (Srayeddin and Doussan, 2009).

Figure 4. Field equipment electrical resistivity tomography

To accurately determine the interactions between soil, plant and atmosphere, it is possible to use different approaches, an example is to estimate the surface energy flows using a two-level model, which considers the flows distributed between soil and vegetation (Norman et al., 1995). The methodology that has developed the most in recent years consists in using micrometeorological measures for the estimation of evapotranspiration flows and, together with measures of the water content of the soil for the estimation of evaporation, measures of lymphatic flow (sap flow) for the estimation perspiration.

The estimation of evapotranspiration can be carried out both through models of considerable diffusion such as that of Penman-Monteith in the version presented in the FAO journal 56 (Allen et al., 1998), and with direct measurements through the use of the eddy covariance technique (EC) (Oishi et al., 2008; Paço et al., 2009; Cammalleri et al., 2013).

3.1 Energy balance

Evapotranspiration is a physical process that requires in addition to the presence of water and a sink (the plant) also that of an energy source, so the estimation of evapotranspiration can also be done through the balance system energy.

The energy balance theory is widely used to study the mass and energy exchanges affecting the soil-plant-atmosphere system.

The evaporation of water requires large amounts of energy, in the form of sensitive heat or radiant energy.

Evapotranspiration is a process governed by the exchange of energy on the surface of the vegetation and is limited by the amount of available energy.

By applying the principle of energy saving it is possible to predict the rate of evapotranspiration.

The incoming energy must be equal to the energy leaving the surface for the same period of time.

All energy flows should be considered when deriving an energy balance equation (**Figure 5**).

The equation for an evaporating surface can be written as:

$$Rn - G - \lambda ET - H = 0 \qquad (9)$$

where Rn (W m^{-2}) is the net radiation, G (W m^{-2}) is the heat flow in the soil (corresponds to about 10% of Rn), λE is the latent heat flow that represents the energy used by the system to evaporate the water and H (W m^{-2}) is the flow of sensitive heat, that is the flow due to the exchange of heat between the surfaces and the atmosphere (Allen et al., 1998).

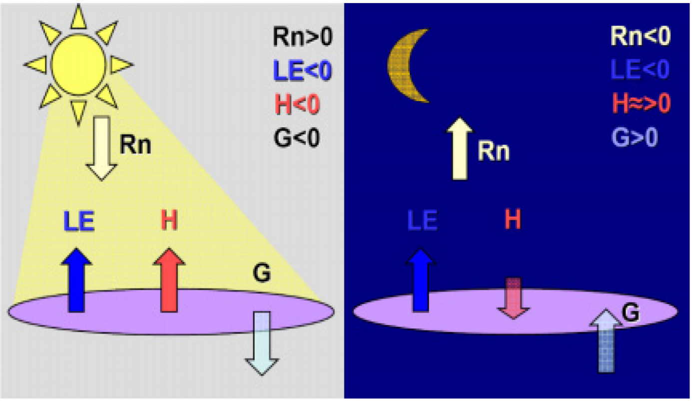

Figure 5. Schematic diagram of the daytime (left) and nighttime (right) energy balance (from Burba and Anderson, 2005).

In equation (9) only vertical flows are considered and the net rate at which energy is transferred horizontally, by advenction, is ignored. Therefore the equation should be applied only to large areas of homogeneous vegetation.

The equation concerns the four components: Rn, λET, H and G. The other energy terms, such as the heat accumulated in the canopy and in the air, the energy required by the plants to the net photosynthesis (<1% of Rn) represent only a small part of the daily net radiation and can be considered negligible when compared with the other four components.

The latent heat flow (λET) which represents the evapotranspiration fraction can be derived from the energy balance equation if all the other components are known. Net radiation (Rn) and soil heat flows (G) can be measured or estimated from climatic parameters. Sensitive heat (H) measurements are however complex and cannot be easily obtained. H requires an accurate measurement of temperature gradients above the surface (Allen et al., 1998).

3.2 Evapotranspiration fluxes

For the management of water resources in agricultural areas, evapotranspiration (ET) plays a role of fundamental importance. From the link between the evapotranspiration flows and the anabolic processes of plants, it follows that these flows are the main regulators of the primary production of any ecosystem (Rallo et al., 2010).

The monitoring and estimation of evapotranspiratory flows can be carried out by means of various techniques and methodologies, the choice of which is often also linked to the environmental conditions that characterize the specific study area.

Through the use of Eddy Covariance stations that allow the measurement of turbulent flows of latent and sensitive heat, radiation net and heat flow in the soil at an agricultural field scale, it is possible to estimate the irrigation requirements of a crop (Corbari et al., 2012).

Although this technique is rather expensive and difficult to apply, it adapts to continuous measurements and over large surfaces and does not disturb the microclimate of the soil (Jassens et al., 2001).

To better understand the Eddy Covariance technique (**Figure 6**) it is necessary to visualize a horizontal air flow that carries within it numerous vortices that rotate, called Eddies, and each vortex has three-dimensional components, including vertical movement, (Burba and Anderson, 2010).

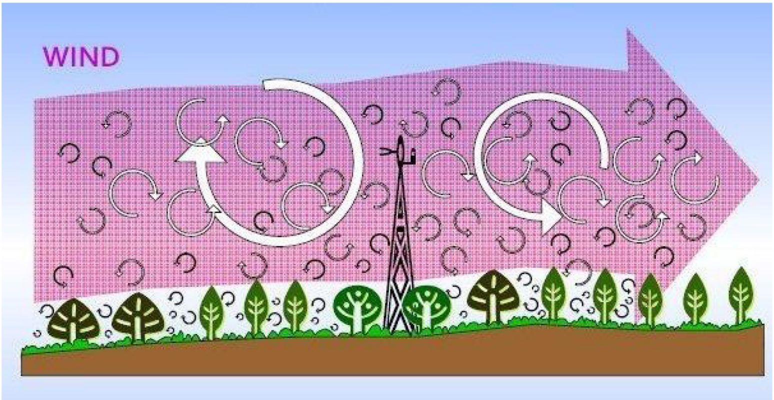

Figure 6. Representation of the flow of air and vortices inside it over a vegetated surface (from Burba and Anderson 2010).

The principle behind the Eddy Covariance is illustrated in **Figure 7**. at a given instant (time 1) an Eddy moves an air packet downwards with a certain speed (w1); at the next instant (time 2) another Eddy moves a packet of air towards the other with speed (w2).

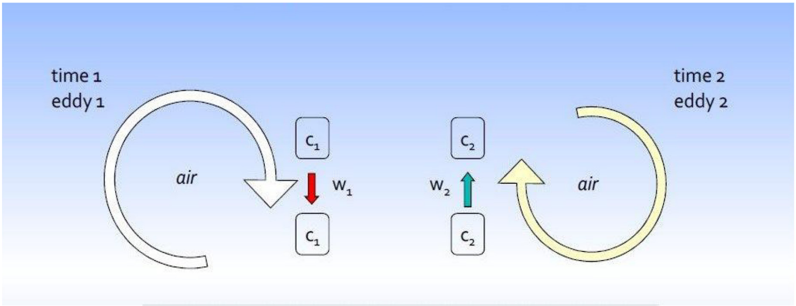

Figure 7. The principle of Eddy Covariance: transport of air packages upwards and downwards (from Burba and Anderson 2010).

Each air package has its own characteristics such as humidity, temperature and gas concentration; being able to measure these characteristics and the vertical speeds of air movements, we thus know the ascending and descending flows of humidity, temperature and gas concentration (Burba and Anderson, 2010).

The instruments used are the gas analyzer and the sonic anemometer which together provide estimates of turbulent flows inside of the surface boundary layer, thanks to the covariance between the vertical wind speed and the water vapor concentration for the latent heat and between the vertical wind speed and the air temperature for the sensitive heat (Corbari et al., 2012).

The instrumentation is mounted on special pylons that overlook the area under study, thus allowing to overhang the atmosphere layer in balance with the vegetation below.

The quality of the data measured with eddy covariance stations is affected by sensor configuration problems, adverse weather conditions such as heavy rainfall or snow and weak turbulence, especially at night, which can lead to errors in the measurement of flows (Foken, 2008; Massman & Lee, 2002). Another fundamental aspect for a good measure is the presence of large and homogeneous surfaces in order to minimize the influence of the surrounding areas, even if this condition is hardly met (Giannico et al., 2018).

The correct functioning of the system is verified by checking the closure of the energy balance (Wilson et al., 2002).

As widely reported in the literature, it is known that this balance never closes because the available energy (net radiation less the heat flow in the soil) is always greater than the sum of turbulent flows (latent heat and sensitive heat) (Corbari et al., 2012; Wilson et al., 2002).

The causes can be traced back to the stability of the atmosphere, in fact during the night, when there are conditions of stable stratification, the flows are generally underestimated due to the low turbulence (Foken, 2008; Massman & Lee, 2002). Therefore, during the night the balance error is greater than during the day (Wilson et al., 2002; Baldocchi et al., 2001).

Another factor to consider when analyzing the balance sheet closure and which can lead to an increase in its closure are attributable to the contribution of additional accumulation flows such as the flow of photosynthesis, the change in enthalpy in vegetation and in the air and the flow of heat in surface soil (Meyers & Hollinger, 2004; Corbari, 2010). Furthermore, the energy balance can be seen as a scale problem, because the representativeness of the measured flows is a function of the scale. It is usually valid to assume that the measurement area is the same for all instruments, but these areas can actually be very different if we compare the footprint of turbulent flows with the measurement area of the heat flow in the soil.

Therefore, the non-closure of the energy balance is partly attributable to the difficulty of matching the footprint area with the measurement area of the instruments that measure net radiation and heat flux in the soil (Wilson et al., 2002; Kustas et al., 2006; Schmid, 1997).

3.3 Transpiration fluxes

The measurement of the plant ranspiratory flows can be indirectly carried out by monitoring some quantities related to the lymphatic flow. The measurement of lymphatic flows is related to the transpiration activity of the plant and consequently to the stomatal conductance, providing a realistic and direct estimate of the water losses of the plant.

The sap flow into the capillaries of a plant's stem can be measured using three different approaches based respectively on the heat pulse velocity (HPV), on the thermal balance, and on the heat dissipation (Smith et al., 1996).

Regarding the HPV technique (**Figure 8**), it consists in inserting inside two holes made in the stem of the plant, in the lower one a heat source and in the upper one a thermocouple. Therefore, this technique is based on the reliefs of the temperature variations produced by a short duration heat impulse (1-2 s), measured in two temperature probes installed asymmetrically on the sides of a heater inserted in the trunk (Sabatti et al., 1992).

Swanson showed that if the temperature rise is measured at distances X_u (m) upstream and X_d (m) downstream from the heater, then the heat pulse velocity can be calculated from:

$$Vz = (X_d + X_u) / 2t_z \qquad (10)$$

tz is the time interval for the temperature difference between the two points considered is equal to zero.

The calculation of Vz from Eq. 10 is based on Marshall's (1958) idealized theory and assumes that heat-pulse probes have no effect on the measured heat flow. Actually, convection of the heat-pulse is disturbed by the presence of the heater and temperature probes, and by the disruption of xylem tissue associated with their placement. These perturbations produce a systematic underestimation in the measured heat pulse velocity (Cohen et al., 1981; Green and Clothier, 1988). Consequently, the heat pulse velocity must be corrected for any probe-induced effects of wounding. This correction can be performed empirically (e.g., Cohen et al., 1981), or based on physical principles, using an equation of the form:

$$V_c=a+bV_z+cV^2_z \qquad (11)$$

where Vc ($m\ s^{-1}$) is the corrected HPV and Vz is the raw HPV given by Eq. 10. The correction coefficients a, b, and c have been derived by Swanson and Whitfield (1981) from numerical solutions of Marshall’s (1958) equations, for various wound sizes.

The speed of sap flow:

$$j=(kF_M+F_L)\ V_c \qquad (12)$$

where F_M and F_L are the volume fractions of wood and water, respectively; k is a coefficient related to the thermal properties of the woody matrix (Becker and Edwards, 1999), and it is assumed to be constant within and between species (Green et al., 2003; Green, 2009).

Figure 8. Heat Pulse Velocity technique. Heat pulse thermistore and heater installed in sapwood of tree

3.4 Physiological plant indices

Water is a fundamebtal resource for the physiology and metabolism of the plant. A lower availability of water resources in plants triggers a series of physiological responses, some of which are used as important indicators of the water status of plants.

Researchers have identified the citrus leaf water potential (Ψ_{leaf}) (de Lima et al., 2015; Rodríguez-Gamir et al., 2010) and stomatal conductance (gs) (Taylor et al., 2015; Villalobos et al., 2009; Jamshidi et al., 2020) the main physiological indicators to reflect the water stress condition of plants.

Leaf water potential expresses the strength with which water is retained by the leaves and it allows to identify when the plant enters water stress condition and therefore to intervene with irrigation.

Generally, the instrument used for the measurement of the leaf water potential is the pressure chamber.

The pressure chamber evaluates the negative hydrostatic pressure (tension) present in the xylem (Taiz e Zeiger, 2002). The use of this technique consists in imposing, on a cut leaf, placed inside the pressure chamber, a pressure increasing by the introduction of gas. After cutting, the water in the capillaries is called inside by the tension that is not counter balanced. In this way it is possible to calculate xilematic water potential since it corresponds in absolute value to that of the leaf.

During the day the water potential can vary significantly as the evaporative demand of the environment changes, the soil water content, the resistance that water encounters passing from the roots to the leaves (Scholander et al.,1965; Xiloyannis, 1992; Remorini and Maasai, 2003; Behboudian and Singh, 2001; Patakas et al., 2005; Girona et al., 2006; Choné et al., 2001; Améglio et al., 1999).

Stomatic regulation is a mechanism that allows the plant to absorb CO_2 during the day and to save water at night, when there is no photosynthesis and therefore a request for CO_2. It plays a fundamental role, since in the presence of water scarcity the closure of the stomata limits the dissipation of heat by transpiration and the leaf tends to heat up and to dissipate sensible heat. Therefore, significant increases in the temperature of the canopy compared to that of the air, are indicative of stomatal closure and therefore of a state of stress. Leaf temperature depends on air temperature, irradiance, wind, humidity and the characteristics of the culture and is measured with an infrared thermometer.

The stomata directly control the transpiration and open or close in response to the concentration of CO_2 in the leaf mesophyll and to the variation of the osmotic potential of the guard cells. They work like hydraulic valves and are sensitive to many factors such as light intensity and quality, temperature, relative humidity and intercellular CO2 concentrations (Taiz and Zeiger, 2002).

Therefore, stomatal control is the main mechanism that regulates water loss in plants.

A very important environmental factor that regulates stomatal functioning in plants and the movement of water from inside the leaf to the outside is the vapor pressure deficit (VPD).

The increase in VPD, due to the increase in air temperature and solar radiation, causes an increase in leaf transpiration, with a consequent lowering of leaf water potential and stomatal closure (Mugnai 2004).

In addition to environmental factors, the increase in soil water restrictions also lead to concomitant reductions in leaf water potential and stomatal conductance (Ballester et al., 2013; Garcia-Tejero et al., 2011; Gasque et al., 2016).

3.5 Bio-chemical plant signals

Plant growth and development are regulated by internal signals and by external environmental conditions. One important regulator that coordinates growth and development with responses to the environment is the sesquiterpenoid hormone abscisic acid (ABA). ABA plays important roles in many cellular processes including seed development, dormancy, germination, vegetative growth, and environmental stress responses in particular it is involved in the response to water scarcity (Endo et al., 2018). These diverse functions of ABA involve complex regulatory mechanisms that control its production, degradation, signal perception, and transduction. (Xiong, et al., 2003; De Menezes de Assis Gomes et al., 2004).

The synthesis of ABA occurs during the drying process and its degradation occurs during rehydration following dehydration (Roychoudhury et al., 2013). For example, the application of the PRD deficit irrigation strategy provides that while one part of the root system is irrigated, the other half is left underground dry; the roots under dry soil trigger a root-to-shoot signaling mechanism based on chemical signals such as abscisic acid (ABA) that are transported to the leaves through transpiration and hydraulic signals (Zhang et al., 1987; Zhang and Davies, 1987; Zhang and Davies, 1989; Zhang and Davies, 1990; Liang et al., 1997; Yao et al., 2001). ABA induces a partial stomatal closure (Zhang and Davies, 1989), resulting in a decrease in leaf transpiration without limiting the assimilation of CO_2 (Jones, 1992), reducing transpiration losses (Davies and Zhang, 1991) without affecting photosynthesis and consequently increasing the watere use efficiency (Dry et al., 1996; Stikic et al., 2003).

Therefore today, ABA is recognized as an important plant hormone that regulates stomatal growth and opening, particularly when the plant is subjected to environmental stress. (Taiz and Zeiger, 2002; Zandalinas et al., 2016).

As a result of water deficit stress the plants respond accumulating non-toxic, low molecular weight organic compounds, known as compatible solutes or osmolytes, such as the amino acid proline (Rhodes, et al., 1994).

The accumulation of proline represents a general response to stress in many organisms, including higher plants, exposed to environmental stresses such as water deficit, high salinity, high temperature, freezing, UV radiation and heavy metals (Delauney, et al., 1993; Siripornadulsil, et al., 2002).

Therefore proline accumulation is protective for plants during different environmental stresses (Hong et al., 2000; Deuschle et al., 2001).

A high proline concentration may maintain the pressure potential under severe deficit conditions due to its potential role as osmoregulator. Proline accumulation is a common response of trees to stress, which can also might contribute in sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth (Blum 1996).

Its accumulation during osmotic stress is mainly due to increased synthesis and is also related with non-hydraulic (chemical) signals as abscisic acid (Conesa et al., 2014).

Several researches have shown a certain proline accumulation in leaves and roots of different species as

Juglans regia (Naser et al., 2010), Sesuvium portulacastrum (Slama et al., 2007), Sativus oryza leaves (Xiong et al. 2012), Arabidopsis thaliana (Sperdouli and Moustakas 2012), Triticum aestivum (Loutfy et al., 2012) during water stress conditions (Zaher-Ara et al., 2016).

In citrus trees, proline accumulation is generally associated to water loss induced by soil water depletion, elevated transpiration rates associated to high temperatures, with different basal levels of proline between genotypes and cultivar (Zandalinas et al., 2016; Hussain et al., 2018).

Therefore, it is necessary to quantify the changes in biochemical mechanisms induced by the application of moderate/severe water deficit conditions also by analysing the role of abscisic acid (ABA) synthesis (Bartels and Sunkar, 2005) and proline accumulation (Moustakas et al., 2011; Zaher-Ara, 2016).

PART II

4. Focus of the thesis

The general aim of the PhD work was to evaluate the efficacy of deficit irrigation (DI) strategies (PRD and RDI) coupled with micro-irrigation techniques (SDI and SSDI) on one of the most important crop species of the Mediterranean region, such as Citrus sinensis L. (cv Tarocco Sciara grafted on Citrange Carrizo). The specific objectives of the PhD work were the following:

- identify the effects of DI strategies on physiological, biochemical and productive plant characteristics;
- evaluate the adequacy of sub-surface drip irrigation methods compared to the most traditional surface drip irrigation;
- individuate the best approach to estimate crop water requirements in terms of crop evapotranspiration (ET_c) and compare the estimates with *in situ* measurements of ET_c, evaluating the impact of deficit irrigation strategies on the performance of the proposed methodologies;
- evaluate the adaptability of the citrus grove following the application of moderate / severe deficit regimes after a decade (from its plantation in 2010).

In order to achieve these objectives, an experimental campaign was carried out during the PhD period.

To present the methodological base of the thesis and the results obtained I chose to collect the articles produced during this PhD period, and to present them in the following four case studies:

- **case study 1** – Authors: Ivana Puglisi, Elisabetta Nicolosi, Daniela Vanella, Angela Roberta Lo Piero, Fiorella Stagno, Daniela Saitta, Giancarlo Roccuzzo, Simona Consoli and Andrea Baglieri; Article title: "Physiological and biochemical responses of orange trees to different deficit irrigation regimes", Plants 2019, 8(10), 423;
- **case study 2** - Authors: Helena Pappalardo, Elisabetta Nicolosi, Daniela Vanella, Biagio Torrisi, Maria Allegra, Fiorella Stagno, Daniela Saitta, Simona Consoli, Giancarlo Roccuzzo, Filippo Ferlito; Article title: "Risposta fisiologica di un agrumeto allo stress idrico controllato", Frutticoltura - n. 1 – 2019 (Physiological response of a citrus grove to controlled water stress);
- **case study 3** - Authors: Daniela Saitta, Daniela Vanella, Juan Miguel Ramírez-Cuesta, Giuseppe Longo-Minnolo, Filippo Ferlito, and Simona Consoli; Article title: "Comparison of orange orchard evapotranspiration by eddy covariance, sap flow and fao-56 methods under different irrigation strategies", Journal of Irrigation and Drainage Engineering, 2020, 146(7): 05020002;
- **case study 4** - Authors: Daniela Saitta, Simona Consoli, Filippo Ferlito, Biagio Torrisi, Maria Allegra, Giuseppe Longo-Minnolo, Juan Miguel Ramírez-Cuesta, Daniela Vanella; Article title: "Adaptation of citrus orchards to deficit irrigation strategies", Agricultural Water Management, 2021, 247, 106734.

Case study 1

Plants 2019, 8(10), 423

5. Physiological and biochemical responses of orange trees to different deficit irrigation regimes

Ivana Puglisi, Elisabetta Nicolosi, Daniela Vanella, Angela Roberta Lo Piero, Fiorella Stagno, **Daniela Saitta**, Giancarlo Roccuzzo, Simona Consoli and Andrea Baglieri

Abstract

The article presents the results of research consisting of the application of deficit irrigation (DI) criteria, combined with the adoption of micro-irrigation methods, on orange orchards (*Citrus sinensis* (L.) Osbeck) in Sicily (Italy) during the irrigation season of 2015. Regulated deficit irrigation (RDI, T3) and partial root-zone drying (PRD, T4) strategies were compared with full irrigation (T1) and sustained deficit irrigation (SDI, T2) treatments in terms of physiological, biochemical, and productive crop response. A geophysical survey (electrical resistivity tomography, ERT) was carried out to identify a link between the percentages of drying soil volume in T4 with leaves abscisic acid (ABA) signal. Results highlight that the orange trees physiological response to water stress conditions did not show particular differences among the different irrigation treatments, not inducing detrimental effects on crop production features. ABA levels in leaves were rather constant in all the treatments, except in T4 during late irrigation season. ERT technique identified that prolonged drying cycles during alternate PRD exposed more roots to severe soil drying, thus increasing leaf ABA accumulation.

Keywords: *Citrus* orchards; crop physiology; irrigation; geophysical surveys; water deficit

Introduction

Citrus species are among the most important tree crops for the Mediterranean agricultural sector. Increasing water use efficiency associated with improved irrigation strategies is a priority for these groves to maintain market competitiveness (Robles et al., 2016). Applied research has already proved that the application of deficit irrigation (DI) strategies for *Citrus* cultivation is effective and sustainable by maximizing water saving without affecting crop yield (Girona et al., 2002) and quality parameters (Carr et al., 2012; Ruíz-Sánchez et al., 2010; García-Tejero et al., 2011; Consoli et al., 2014, 2017; Pérez-Pérez et al., 2018). Nevertheless, it is necessary to quantify the changes in biochemical mechanisms induced by the application of moderate/severe water deficit conditions by analyzing the role of abscisic acid (ABA) (Bartels et al., 2005) and proline accumulations (Moustakas et al., 2011; Zaher-Ara et al., 2016). In fact, ABA is recognized as an important stress-signaling hormone, acting in the regulation of stomatal closure, synthesis of compatible osmolytes, and in the upregulation of genes leading to adaptive responses (Bartels et al., 2005). Among osmolytes, proline is considered an active molecule and its accumulation is putatively mediated by free radicals produced as a result of oxidative stress (Moustakas et al., 2011; Zaher-Ara et al., 2016). Proline accumulation plays a protective role for plants in the face of different environmental stresses (Hong et al., 2000; Deuschle et al., 2001). In *Citrus* trees, proline accumulation is generally associated with water loss induced by soil water depletion, elevated transpiration rates associated with high temperatures, and with different basal levels of proline between genotypes and cultivar (Zandalinas et al., 2016; Hussain et al., 2018).

With respect to the existing literature, the innovative aspects introduced in this study concern the investigation of the effects of DI strategies application on biochemical and physiological responses of mature *Citrus sinensis* (L.) Osbeck crops. The specific objectives of the study are: (i) to assess the sustainability of partial root-zone drying (PRD), regulated deficit irrigation (RDI), and sustained deficit irrigation (SDI), integrated with surface and sub-surface micro-irrigation techniques, in maintaining adequate physiological and productive crop responses; and (ii) to identify links between the quota of water deficit and physiological and biochemical plant responses.

Materials and methods

Experimental Site, Climatic Data, and Crop Water Demands

The study was carried out in a 1 ha experimental field in Eastern Sicily, Italy (latitude 37°20′ N, longitude 14°53′ E; 50 m altitude) where orange trees *cv* Tarocco Sciara grafted on Carrizo citrange, (*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck) were planted in 2010, with a between-row spacing of 6 m and a within-row spacing of 4 m. The crops have undergone DI regimes since the youth phase (i.e., the year 2010). The experiment was set as a randomized block design with three irrigation treatments, replicated three times (Consoli et al., 2014): (i) Full irrigation (control, T1), 100% crop evapotranspiration (ET_c) using a surface drip irrigation system; (ii) sustained deficit irrigation (SDI, T2) irrigated at 75% ET_c using a subsurface drip irrigation system; (iii) regulated deficit irrigation (RDI, T3) irrigated at

100% ET_c, except in II phenological stage (i.e., fruit growth, at 50% ET_c), using a surface drip irrigation system; and (iv) partial root-zone drying treatment (PRD, T4) irrigated at 50% of ET_c where the water was supplied by two drip lines placed respectively at the eastern and western side of the plants, and used alternatively every 14 day intervals. Each treatment consists of three rows of eight trees, for a total of 24 plants (**Figure 1**).

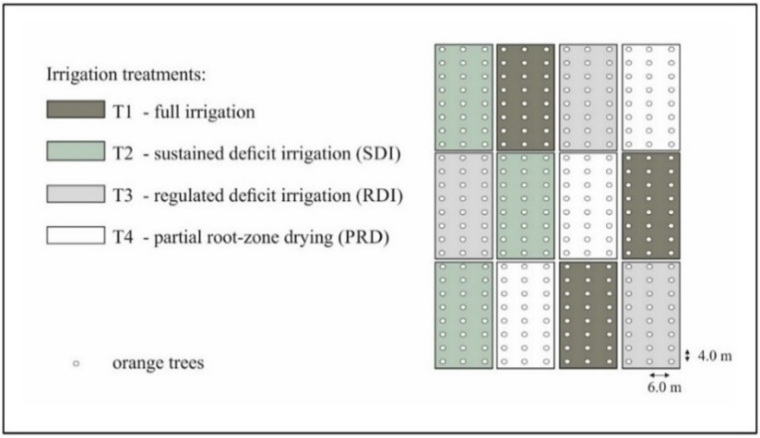

Figure 1. Lay-out of the deficit irrigation (DI) experimental site (Eastern Sicily, Italy).

Irrigation was applied during the irrigation season 2015, from mid-June (DOY 166) to mid-October (DOY 289), three times per week, early in the morning.

An automatic weather station, located at the farm, registered hourly meteorological data (i.e., solar radiation, R_s, W m^{-2}, air temperature, T_{air}, °C, relative humidity, RH, %, wind speed, u, ms^{-1}, and direction of rainfall), which were then used to calculate reference ET (ET_0 mm d^{-1}) through the

Penman–Monteith approach (Allen, R.G et al., 1998; Allen, R.G et al., 2006). Crop evapotranspiration (ET_c) was obtained by multiplying daily ET_0 by the seasonal crop coefficient (K_c) for orange orchard (i.e., 0.7) as assessed by Consoli et al. (2006,2013). Correction coefficients were applied to K_c to consider canopy size (i.e., 0.65), irrigation method efficiency (i.e., 0.9), and the occurrence of rainfall events.

Measurements of transpiration (T_{SF}) at tree level were obtained by the heat pulse velocity (HPV) technique (Swanson et al., 1981), which is based on the measurement of temperature variations (ΔT) produced by a heat pulse of short duration (1–2 s). The measurements were taken in two temperature probes installed asymmetrically on either side of a linear heater inserted into the trunk. In particular, one 4 cm sap flow probe, with two embedded thermocouples (Tranzflo NZ Ltd., Palmerston North, NZ), was positioned in the trunks of the trees (i.e., at south side of the trunk, 20 cm from the ground) and wired to a data-logger (CR1000, Campbell Sci., Logan, UT, USA) for heat-pulse control and measurement; the sampling interval was set at 30 min. Data were processed according to Green et al. (2003) to integrate sap flow velocity over sapwood area (determined as reported in Consoli et al. (2017)) and calculate transpiration fluxes.

Irrigation water had electrical conductivity (EC 25 °C) of 2.02 dS m^{-1} (medium salinity) and pH of 7.30. The soil volumetric water content (θ_v) was measured using 10 ECH_2O probes (Decagon, Inc., Pullman, WA, USA) located at different depths (0.15–0.40 m) of the irrigation treatments. The amount of available water (AWA) was calculated according to the following equation:

$$\mathrm{AWA} = \frac{\theta_v - \theta_{WP}}{\theta_{FC} - \theta_{WP}}, \tag{1}$$

where θ_v (m^3 m^{-3}) is the actual soil water content, θ_{WP} (m^3 m^{-3}) is the soil water content at the wilting point, and θ_{FC} (m^3 m^{-3}) is the soil water content at the field capacity. The soil at the experimental site resulted fairly uniform, with a sandy-loam texture (69.7% sand, 10.5% clay, 19.8% silt), mean θ_{FC} (pF = 2.5) and θ_{WP} (pF = 4.2) of 24% and 14%, respectively (Aiello et al., 2014; D'Emilio et al., 2018). Soil samples were collected at depths between 0.05 and 0.25 m for physical–chemical laboratory determinations, air-dried, and then sieved at 2 mm. Organic carbon (OC), nitrogen (N), cation exchange capacity (CEC), Ca^{2+}, Mg^{2+}, K^+ and Na^+ exchangeable elements, available phosphate (P), electric conductivity, and pH were determined according to Page et al. (1982) and following the Italian Ministerial Decree (MD) 13/09/1999.

ABA and Proline Content Detection in Orange Leaves

Trees leaves (1 g) were randomly sampled from the four treatments subjected to different irrigation treatments, frozen in liquid nitrogen, and stored at –80 °C until further laboratory analysis. The abscisic acid (ABA) concentration was determined with a Phytodetek ABA enzyme immunoassay test kit (Agdia, Elkhart, IN, USA), according to the manufacturer's protocol. The frozen leaves were ground into powder and homogenate in 10 mL of 80% acetone, 0.5 g L^{-1} citric acid, and 20 mg L^{-1} butylated hydroxytoluene (Hubick et al., 1980). The suspension was centrifuged at 3000 × g for 5 min, and the supernatant was diluted with Tris-buffered saline (45 mM Tris-HCl, pH 7.8, 90 µM $MgCl_2$, 0.135 M NaCl, and 3 mM sodium azide).

Samples were subdivided into two fractions to determine both free and total ABA. To determine the total ABA, the hydrolysis of ABA glucosyl ester (ABA-GE) was performed by adding 0.1 M sodium hydroxide and incubating in a water bath at 60 °C for 1 h. Then, samples were cooled in an ice bath and pH was adjusted by chlorhydric acid. The absorbances were detected at 405 nm. The ABA concentration was determined from a standard curve. ABA-GE was calculated by subtracting free ABA to total ABA.

Proline was determined spectrophotometrically following the ninhydrin method of Bates et al. (1973) modified by Khedr et al. (2003). Briefly, the frozen citrus leaves (1 g) were homogenized in 3% aqueous sulphosalicylic acid and the residues were removed by centrifugation at 12,000 × g for 10 min. The supernatant (1 mL) was mixed with 1 mL of glacial acetic acid and ninhydrin reagent in a 1:1 (v/v) ratio. The reaction mixture was incubated at 100 °C for 1 h. After extraction with toluene, the absorbance of the organic phase was read at a wavelength of 520 nm, using toluene as a blank. The proline concentration was determined from a standard curve using D-proline.

Plant Physiological Indicators and Productive Crop Features

Stem water potential (Ψ_S) was measured at midday with a pressure chamber (SKPM 1405/40, Skye Instruments, Llandrindod Wells, UK), as described by Scholander et al. (1965) and following the procedure reported in Turner (1981). For each treatment, two leaves from four different trees of each replica (total of 24 leaves for each treatment) were monitored. Measurements were carried out on fully exposed sunlight leaves, bagged in plastic bags, and covered with silver foil at least 1 h prior to determinations. Stomatal conductance (g_s, mmol m^{-2} s^{-1}) was obtained using a leaf porometer (Decagon Devices Inc., Pullman, WA, USA) during the central hours of the day (between 11:00 and 13:00). Measurements were performed in six fully exposed leaves per tree and four trees per treatment. Leaf stomatal density was determined using the impression approach (Radoglou et al., 1990; Binley et al., 2015), which expresses the number of stomata per unit leaf area (opened and closed stomata). The impression was taken from the surface of around 0.02 m^2 of fully expanded leaves in the mid-area between the central vein and the leaf edge. The thin film was peeled off from the leaf surface and the number of stomata was counted by using an image analyzer (Leica ASM 68 K) and the software Image Tool. Leaf area index (LAI, m^2 m^{-2}) and photosynthetically active radiation (PAR, %) were monitored at plant level with a ceptometer (Accu PAR LP-80, Decagon Devices Inc., Pullman, WA, USA). Fresh and dry weights (g) were obtained on five leaves per tree per irrigation treatments. Leaf dry weight was obtained by drying leaves in an oven at 75 °C until constant weight was reached. Total yield (t ha^{-1}) and fruit weight (g) were determined at time of commercial harvest in February 2016. Ten fruits were chosen from 12 trees per treatment and replication and were analyzed for determining the equatorial section (ED, mm) through a caliber tape.

The use of Electrical Resistivity Tomography (ERT) to identify soil drying pattern under PRD

Soil electrical resistivity (ER) distribution represents an indirect indication of the soil water state (e.g., porosity, water content, and pore water salinity) (Consoli et al., 2017).

Electrical resistivity tomography (ERT—see Binley et al. (2015), among others) consists of the injection of an electrical current in the subsoil by a pair of electrodes and the subsequent measurement of the electrical potential. This acquisition is repeated through many combinations of transmitting and receiving electrodes in order to acquire data that can then be inverted to produce two-dimensional (2-D) or three-dimensional (3-D) images of ER distribution on the subsurface. The use of borehole electrodes enhances resolution at depth. In this study, small-scale 3-D ERT monitoring was conducted around 2 selected orange trees irrigated at full level (T1) and by PRD (T4). For each tree, the setup consists of 6 boreholes (1.2 m deep,) each housing 12 electrodes (vertically spaced 0.1 m), plus 48 surface electrodes (spaced 0.26 m on a regular square grid) (details in Vanella et al. (2018,2019)). The ERT setup covered a soil volume of about 4 m^3 (1.3 × 2.6 × 1.2 m). The 3-D ERT monitoring was conducted during the mid and at end of the irrigation season 2015 (DOYs 195–264). For each 3-D ERT monitoring, two datasets were acquired using a Syscal Pro Switch 72 resistivity meter (IRIS Instruments, Orléans, France), one related to the initial condition, to be used as background dataset, and the one after the irrigation phase (time-lapse mode). A total of 8 drippers were located at the surface of the control volume in T1 and T4. In T4, irrigation was supplied by the active pipeline located on the east or west sides of the tree trunk and lasted about three hours. Data quality was assessed using a full acquisition of reciprocals to estimate the data error level (see Binley et al. (2015), amongst many others). The estimation of the ER as a percentage of the background ER was obtained by 3-D data inversion using the

Occam approach as implemented in R3t software package (Binley 2019).

Statistical Analysis

The acquired data were subjected to one-way analysis of variance (ANOVA) (Statistica 6.0 package, Statsoft Inc., Tulsa, OK, USA). A fixed factor corresponding to the four-level irrigation treatment, T1, T2, T3, and T4 (randomly distributed at the experimental site under study), was used for analyzing the physiological and biochemical responses of orange trees to the different deficit irrigation regimes. In the case of significant difference (*p* value < 0.05), means were separated using the Tukey's test.

Results

Soil Characteristics and Plant Physiological Response to Deficit Irrigation Strategies

Climate conditions during 2015 were typical of Mediterranean semi-arid regions, with hot and dry summers. During the irrigation season (June to October 2015), the maximum air temperature (T_{air}) reached 30 °C, with mean values of vapor pressure deficit (VPD) and relative humidity (RH) equal to 0.62 kPa (**Figure 1**) and 69.8%, respectively.

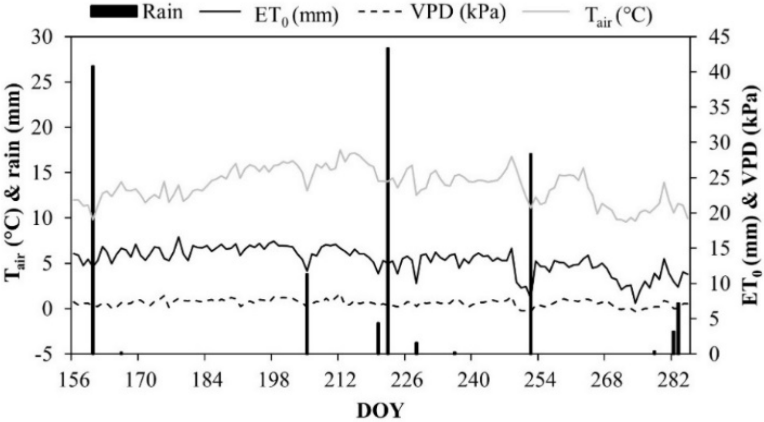

Figure 2. Air temperature (T_{air}), vapor pressure deficit (VPD), reference ET (ET_0), and rainfall at the experimental site during the irrigation season 2015.

The reference evapotranspiration (ET_0) had a mean of 5.4 mm d^{-1}, reaching a total of around 700 mm. ET_c was about 300 mm and rainfall was less than 100 mm. The amount of irrigation (mm) supplied at the different treatments and the corresponding water savings are reported in **Table 1**.

Table 1. Irrigation rates and water savings for the different treatments (T1, control; T2, SDI; T3, RDI; T4, PRD) at the experimental site during irrigation season 2015.

	T1	T2	T3	T4
Irrigation Rates (mm)	279.0	204.2	174.2	158.0
Water Savings* (%)	--	26.8	37.6	43.4

*(1- (irrigation T_i = 2, 3, 4 / irrigation T1)) × 100).

The water deficit (%), shown in **Table 1**, was only slightly different from those theoretical fixed for each treatment, thus demonstrating the adequacy of the irrigation system setup. Table 2 reports the main physical and chemical soil characteristics for each of the different treatments. Cation exchange capacity (CEC), electrical conductivity (EC), and pH did not show any differences among the treatments.

Table 2. Chemical and physical soil properties at the different irrigation treatments in 2015.

Treat.	**OC** **(g kg^{-1})**	**N_{TOT}** **(g kg^{-1})**	**CEC** **(meq 100g^{-1})**	**Ca** **(g kg^{-1})**	**Mg** **(g kg^{-1})**	**K** **(g kg^{-1})**	**Na** **(g kg^{-1})**	**P_{avail}** **(mg kg^{-1})**	**EC** **(mS cm^{-1})**	**pH**
T1	10.8 a	0.6 b	48.9 a	5.2 b	2.9 a	1.2 b	1.8 a	56.0 a	0.32 a	8.1 a
T2	10.3 a	0.3 c	43.3 a	7.8 a	1.7 b	1.0 b	0.9 b	42.9 ab	0.26 a	8.3 a
T3	12.7 a	1.6 a	46.1 a	5.8 b	2.1 ab	1.9 a	1.3 b	44.9 ab	0.27 a	8.3 a
T4	8.8 b	0.8 b	40.0 a	4.9 b	2.7 ab	1.9 a	1.6 a	39.9 b	0.25 a	8.5 a

OC: Organic carbon; CEC: Cation exchange capacity; EC: Electrical conductivity. Values in the same column followed by different letters were significantly different ($p < 0.05$).

Soil in T4 (PRD) showed lower organic carbon (OC) content than the other treatments, whereas T3 (RDI) showed higher N_{TOT} values. The highest available phosphate content (P) was observed in T1 and the lowest in T4.

The amount of available water (AWA) in the investigated soil profile was maintained around 80% in T1, 71% in T2, 58% in T3, 43% in T4 East, and 46% in T4 West (**Figure 3**).

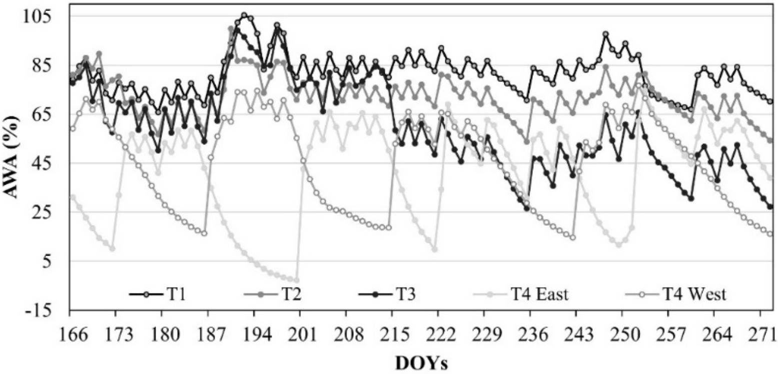

Figure 3. Seasonal evolution of the amount of available water (AWA) in the soil profile of the DI treatments. DOY: Day of the year.

In T1, T2, and T3, the soil volumetric water content (θ_v) remained very close to field capacity (θ_{FC}) condition, while θ_v in T4 was characterized by the expected alternation between drying and wetting cycles (T4, East and West), decreasing slightly below the threshold of the wilting point (θ_{WP}) (minimum θ_v value of 0.11 cm^3 cm^{-3}). **Figure 4** reports the cumulative plant transpiration (T_{SF}) for T1 and T4, obtained with heat pulse method (HPV) method.

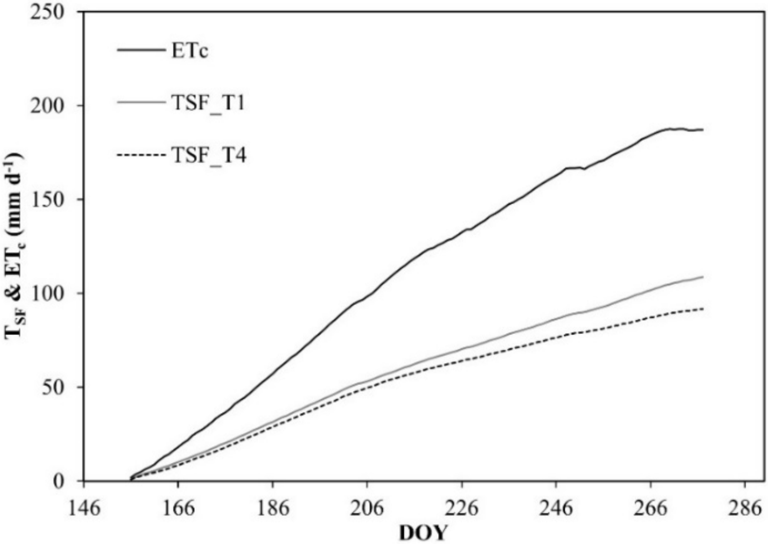

Figure 4. Cumulative values of T_{SF} and ET_c rates in T1 and T4. DOY: Day of the year.

Results evidenced that T4 transpired 92 mm during the irrigation season, about 58% less than ET_c, while T1 transpired about 49% less than ET_c. The discrepancy between T_{SF} rate in T1 and crop evapotranspiration (ET_c) was mainly due to soil evaporation. As reported in Table 3, fresh and dry weights of the leaves were similar among the different irrigation treatments during the monitoring.

Table 3. Physiological plant response indicators to DI. Values (average and standard deviation) of the same columns for each DOY followed by different letters were significantly different ($p < 0.05$).

DOY	Treatment	Fresh Weight (g/leaf)	Dry Weight (g/leaf)	Total Stomata (number/m^2)	Opened Stomata (%)	LAI (m^2 m^{-2})	PAR (%)
174	T1	0.8 ± 0.2 b	0.3 ± 0.1 b	372.3 ± 52.2	66.6	4.7 ± 0.9	71.5 ± 4.6
	T2	1.1 ± 0.3 a	0.4 ± 0.1 a	369.3 ± 51.0	77.9	5.2 ± 0.3	75.2 ± 2.8
	T3	0.9 ± 0.2 ab	0.3 ± 0.1 ab	358.9 ± 60.8	68.5	5.5 ± 0.8	75.3 ± 2.0
	T4	0.9 ± 0.3 ab	0.3 ± 0.1 ab	382.4 ± 51.2	69.9	5.0 ± 0.7	73.4 ± 3.6
215	T1	1.1 ± 0.3	0.4 ± 0.1	328.8 ± 51.0	36.6	4.0 ± 0.6	69.5 ± 6.3
	T2	1.8 ± 0.4	0.4 ± 0.2	294.6 ± 77.9	31.1	4.3 ± 0.3	74.8 ± 1.7
	T3	1.1 ± 0.2	0.4 ± 0.1	320.1 ± 90.1	39.7	4.5 ± 0.7	79.1 ± 7.1
	T4	1.2 ± 0.2	0.5 ± 0.1	344.4 ± 61.1	38.9	4.9 ± 0.3	80.4 ± 13.3
244	T1	1.2 ± 0.4	0.4 ± 0.14	279.0 ± 50.8 ab	36.8	3.4 ± 0.5	69.1 ± 8.2
	T2	1.1 ± 0.3	0.4 ± 0.14	260.5 ± 54.0 b	24.8	4.1 ± 0.6	74.7 ± 1.3
	T3	1.1 ± 0.3	0.4 ± 0.10	324.3 ± 53.4 a	35.9	3.3 ± 0.7	70 ± 3.8
	T4	1.1 ± 0.2	0.4 ± 0.11	333.0 ± 56.9 a	27.8	3.6 ± 0.7	69.3 ± 9.6
272	T1	1.6 ± 0.3 a	0.6 ± 0.1 a	356.6 ± 39.4 ab	61.5	2.2 ± 0.3	44.7 ± 9.6
	T2	1.2 ± 0.2 b	0.5 ± 0.1 b	354.6 ± 48.5 b	75.5	2.6 ± 0.9	54.4 ± 12.2
	T3	0.9 ± 0.2 b	0.4 ± 0.1 b	405.9 ± 70.7 a	56.5	2.4 ± 0.3	53.7 ± 11.3
	T4	0.9 ± 0.2 b	0.4 ± 0.1 b	397.8 ± 78.8 ab	63.1	2.0 ± 0.5	43.3 ± 6.5

As an exception, a slight increase of the T2 indicators compared to T1 was detected at the beginning of the irrigation season and at the end of September (day of the year (DOY) 272). The open stomata were fairly constant, while the total stomata decreased slightly in T2. Leaf are index (LAI) and photosynthetically active radiation (PAR) were fairly similar among the different treatments during the monitoring, with the exception of a certain reduction after DOY 174, due to pruning. Total yield and equatorial section (ED) did not show any difference among the DI treatments and T1 (**Table 4**).

Table 4. Plant production characteristics at harvest (February 2016). Values refer to average and standard deviation.

Treatment	**Mean Fruit Weight (g)**	**Total Yield ($t\ ha^{-1}$)**	**Equatorial Section (ED mm)**
T1	259.3 (± 7.6)	24.6 (± 1.78)	77.3 (± 0.78)
T2	264.8 (± 11.1)	22.7 (± 1.38)	78.7 (± 1.46)
T3	276.0 (± 10.4)	23.5 (± 2.48)	79.2 (± 1.09)
T4	253.0 (± 12.0)	24.7 (± 3.80)	77.6 (± 1.30)

The values of stem water potential (Ψ_{stem}) resulted fairly similar during the monitoring and among the different treatments. The lowest values (–1.8/–2.2 MPa) were recorded at DOY 160, before the beginning of the irrigation season (DOY 166 to DOY 289). In all the treatments, Ψ_{stem} ranged between –1.4 and –2.2 MPa (**Figure 5**).

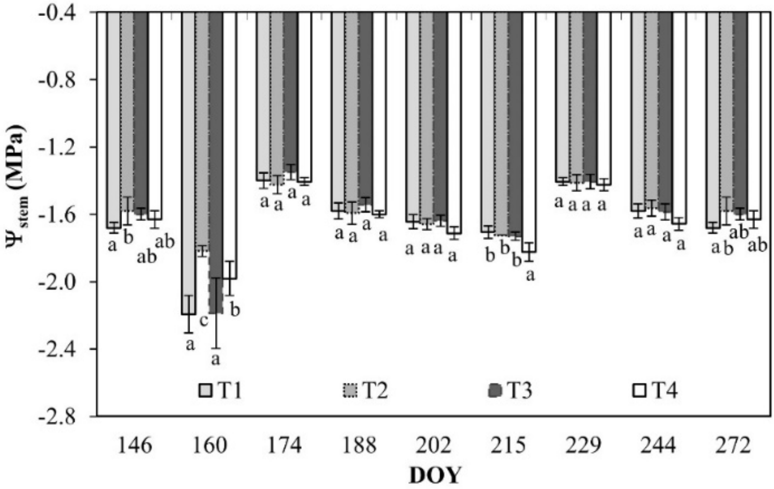

Figure 5. Stem water potential (Ψ_{stem}) for leaves of orange trees at the different irrigation treatments. Vertical bars are standard deviation. For each date, the average values that share a letter are not statistically different. DOY: Day of the year.

During the monitoring, g_s varied from a maximum of about 224 mmol m^{-2} s^{-1} in T1 to a minimum of 40 mmol m^{-2} s^{-1} in T4 (**Figure 6**).

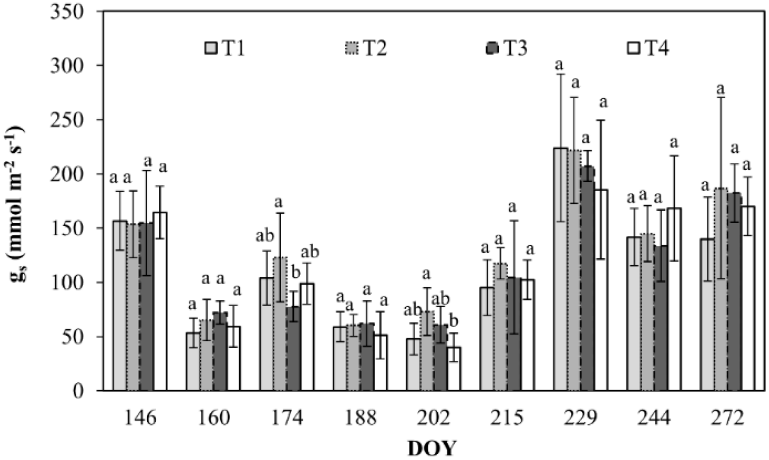

Figure 6. Stomatal conductance (g$_s$) for sunny leaves of orange trees at the different irrigation treatments. Vertical bars are standard deviation. For each date, the average values that share a letter are not statistically different. DOY: Day of the year.

ABA and Proline Detection in Orange Leaves

Total ABA, free ABA, and ABA-GE contents in leaves are reported in **Figure 7a–c**, respectively.

During DOY 272 (late irrigation season), a sharp increase in free ABA was registered in T4 (PRD). During the monitoring period, ABA components did not show differences among the irrigation treatments; only during DOY 215 free ABA of RDI leaves (T3) increased compared to the other treatments (**Figure 7b**). ABA-GE was generally higher in T1 (**Figure 7c**) and at DOY 215 it was not detected in treatments T3 and T4. At the end of the irrigation season, in all the investigated treatments, ABA-GE was completely hydrolyzed into free ABA to contrast water stress conditions.

Proline content in leaves of all the irrigation treatments increased during the monitoring period, reaching a maximum at DOY 272 (**Figure 8**) for T1 and T2. At the end of irrigation season, T1 and T2 treatments increased their proline content, respectively, 3.7-fold and 6.8-fold compared to previous values (DOY 244). At DOY 215, T1 resulted to be higher than other treatments, whereas at DOY 244, T4 showed values of proline around twice as high than others.

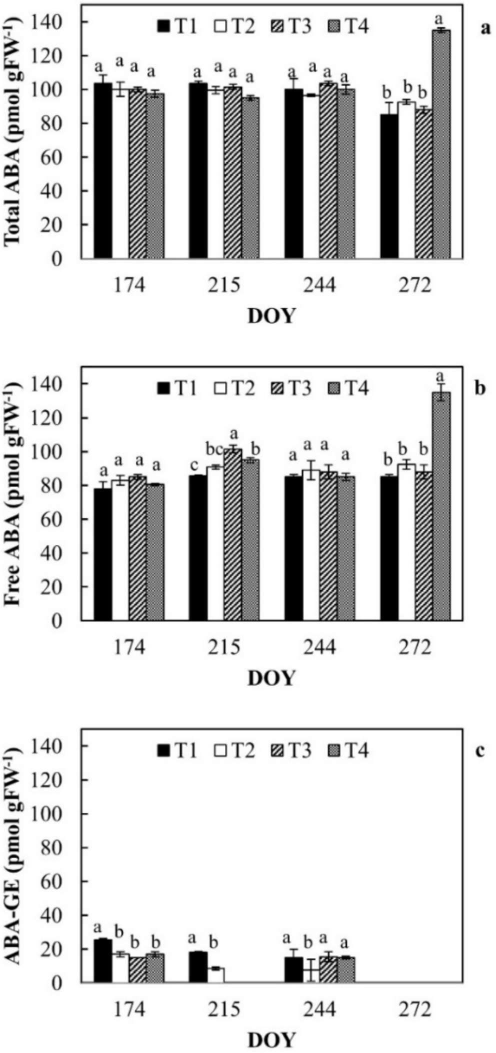

Figure 7. Total ABA (a), free ABA (b) and conjugated ABA (c) in leaves of orange trees at the different irrigation

treatments. For each date, the average values that share a letter are not statistically different. DOY: Day of the year.

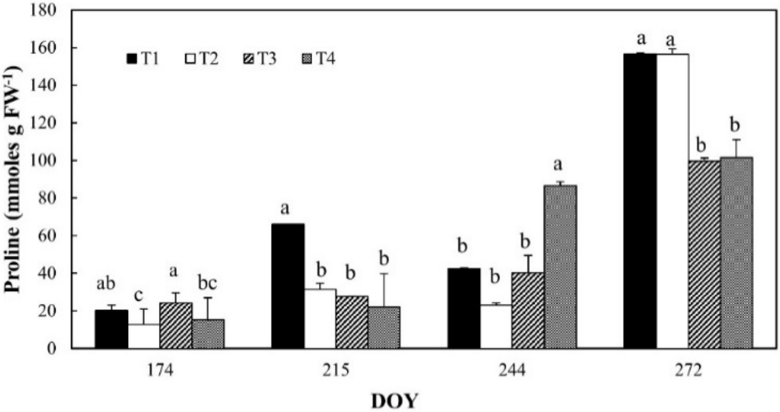

Figure 8. Proline content in leaves of orange trees at the different irrigation treatments. For each date, the average values that share a letter are not statistically different. DOY: Day of the year.

The Use of ERT to Identify Soil Drying Pattern under PRD

Figure 9 shows the % of increase (ration higher than 100%) or decrease (ratio lower than 100%) of electrical resistivity (ER) in the investigated soil volumes in T1 and T4, during the electrical resistivity tomography (ERT) surveys, compared to the initial conditions (background, ratio of 100%), when no irrigation was applied.

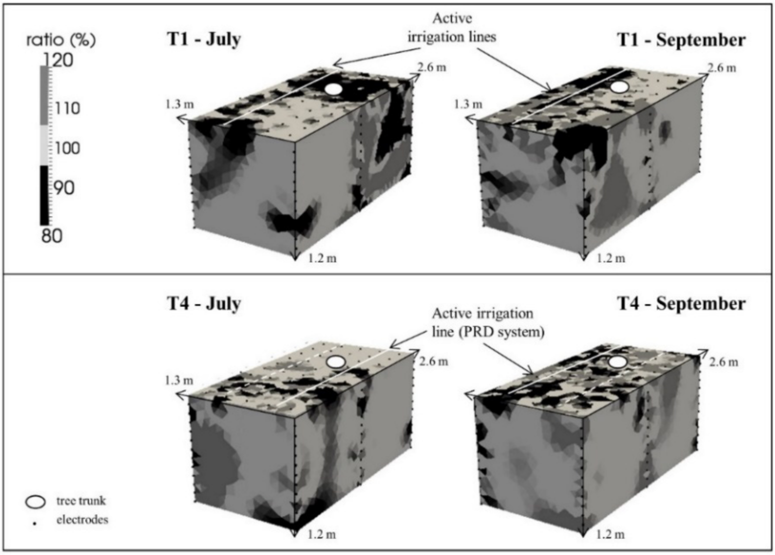

Figure 9. Electrical resistivity (ER) changes on the investigated soil volumes in T1 and T4.

The main effects of the simultaneous phenomena occurring within the soil of T1 and T4 (i.e., infiltration and root water uptake) were wetting (ER decrease) and drying (ER increase) patterns. At the end of the irrigation phase, about 35% (± 9.1%) of the soil volume in T1 presented a marked decrease in the ER due to the progression of the infiltrated irrigation front. At the same time, on average, 13% (± 13%) of soil volume in T4 was increased by wetting patterns, with a decrease in ER. Recognizable soil drying pattern, corresponding to an increase in ER values, interested on average more than 25% (± 10%) of the soil volume in T4 and less than 15% (± 5%) of the soil volume in T1. **Figure 10** shows the comparison between free ABA accumulation and the % of decreasing ER in the soil volumes in T1 and T4.

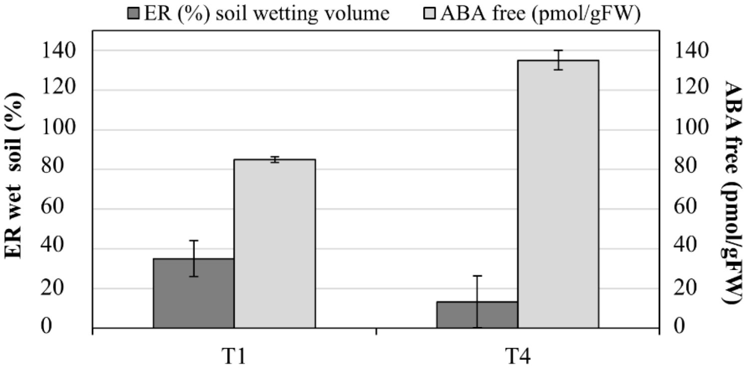

Figure 10. Free abscisic acid (ABA) accumulation in leaves (pmol gFW^{-1}) and % of wetted soil by electrical resistivity tomography (ERT) in T1 and T4.

Discussion

The results obtained during the research encourage the adoption of DI criteria for high-value Mediterranean crops, like orange orchards, particularly susceptible to the occurrence of climatic change scenarios. This study confirms and builds upon previous research carried out on the same issue by the same authors in the same study area (Consoli et al., 2014, 2017; Stagno et al., 2015). Additionally, it introduces new observations while identifying new possibilities of irrigation for the minimization of the negative effects of severe water deficit (i.e., 50% of ET_c in T4).

First of all, this study shows that it is possible to maintain soil fertility even in severe deficit irrigated treatments, as already shown in different works (Farooq et al., 2009). As a matter of fact, despite its lower OC with constant and equilibrate N_{TOT}

(Stevenson et al., 1994), T4 sustained yield. This can be explained by previous studies, which demonstrated that the effects on soil physical–chemical composition affecting root water uptake, under severe and prolonged water deficit conditions but frequent soil rewetting, allow partial compensation of induced side effects (Austin et al., 2004; Borken et al., 2009).

Second, the study provides useful information on the various irrigation methods, in particular on surface drip irrigation vs. sub-surface drip irrigation, or T1 vs. T2. The SDI (T2) treatment, by eliminating about 25% of water losses for evaporation, is quite similar to T1. As suggested by Consoli et al. (2014,2017) and García-Tejero et al. (2011), the plants in T1 and T2 had similar physiological, biochemical, and productive responses.

Third, the study gives useful information about the physiological response (i.e., Ψ_{stem}, g_s, and stomata open/closure) of the deficit irrigated orange orchards. The physiological indicators are very sensitive in these kinds of studies; the response to deficit conditions is snap and show coherence between stem water potential and stomata conductance. As expected, the most negative Ψ_{stem} of about –1.8 MPa was recorded for T4 in the middle of the irrigation season. Generally, values of Ψ_{stem} and g_s are consistent with moderate water stress conditions, as confirmed by several studies (Consoli et al., 2017; Ortuño et al., 2006; Capra et al., 2008; Damour et al., 2010). Opened stomata (%) as well as total stomata (number) were not influenced by water deficit conditions (**Table 3**), although Xu and Zhou (2008) and Damour et al. (2010) found a certain degree of leaf trait plasticity (i.e., determining adaptation) in response to environmental changes, including water stress conditions (Ahmad et al., 2016). Plants in T4 acquired a stomatal control mechanism and lower water content in the soil that allowed them to regulate the T_{SF} mechanism on the released water availability (T_{SF} in T4 is about 15% less than in T1).

The synchronization of stomatal resistance and Ψ_{stem} may occur due to a hydro-active, negative feedback response, involving a biochemical- (e.g., proline) and hormonal- (e.g., ABA) mediated response of guard cells to perturbations of the leaf water potential or hydro-passive (Steppe et al., 2006). In our case, the Ψ_{stem} changes were not associated with proline accumulation, suggesting the hypothesis of a physiologic accumulation of this osmolite, as confirmed by the literature (Hanson et al., 1994; Ben Hassine et al., 2008; Parida et al., 2008; Hayano-Kanashiro et al., 2009; Mattioli et al., 2009). Some authors have shown that the proline accumulation can occur in physiological conditions related to growth purposes, since a significant amount of this amino acid increased its concentration in the reproductive organs of different non-stressed plant species (Mattioli et al., 2009; Chiang et al., 1995; Schwacke et al., 1999). This is in line with the results obtained in this study in T1 and T2 treatments of DOY 272.

Fourth, this study involved an interesting aspect regarding the ABA contents found in leaves of the different irrigation treatments. Generally, in experimental open-field conditions like ours, numerous abiotic stress conditions co-occur simultaneously, producing a unique plant response. For example, as suggested by Zandalinas et al. (2016), while water stress could induce ABA accumulation in citrus tissues, heat stress may inhibit ABA accumulation; thus, stressed citrus leaves may undergo substantially different programs regulating ABA homeostasis.

Under water stress conditions, apoplastic pH increases resulting in greater retention of ABA, functioning as a signal to reduce transpiration in leaves (Finkelstein et al., 2013). Endogenous free ABA levels are regulated through the coordinated action of biosynthesis, catabolism, and conjugation that mainly produces ABA-GE, which is considered one of the major inactive forms of ABA (Finkelstein et al., 2013; Hartung et al., 2002). Recently, Romero et al. (2012) found that in *Citrus sinensis* L. Osbeck, the response to moderate dehydration in ABA-deficient mutant included both ABA-dependent and independent pathways. Accordingly, our results support the hypothesis that, in orange trees, an ABA-independent pathway regulates the stress response in field, where different stressing factors along with water deficit irrigation occur.

Moreover, our results showed that, with the exception of PRD (T4), all the investigated DI treatments, if compared to the control, do not induce any ABA stress signaling involved in an adaptive response. The effect of this increase of ABA levels at T4 can putatively trigger a later adaptive response, as suggested by Romero et al. (2012). In fact, at the end of September, plants reset their ABA-GE reserve, making it all available.

Finally, recent studies indicate that prolonging the drying cycles during alternate PRD exposes more roots to severe soil drying, increasing root and leaf ABA accumulation, and enhancing crop yields and quality (Pérez-Pérez et al., 2018). As in our study case, leaf ABA accumulation was registered in the DI treatment, which substantially decreased the transpiration rate (PRD transpires about 44% less than ET_c) (Puértolas et al., 2014). These observations are consistent with a model that explains leaf free-ABA concentration of PRD plants as a function of xylem ABA concentrations emanating from the irrigated and drying parts of the root system and the relative sap flow from each plant (Dodd et al., 2008; Pérez-Pérez et al., 2015). Moreover, in our study, an inverse relationship was observed between free ABA concentrations and soil wetting dynamics by ERT (**Figure 10**), confirming that, as evidenced by Pérez-Pérez et al. (2018), prolonged exposure of half of the root system to drying soil combined with alternate re-watering can improve ABA accumulation (169.96 ± 24.83 pmol gFW^{-1} at the end of the irrigation season in T4).

Conclusions

The challenge for agriculture in the near future will be to combine water use efficiency with increased resilience in all the productive systems. In this view, the study herein presented focuses on the feasibility of the application of moderate (RDI) and severe (PRD) water deficit conditions to high-value cropping systems, like orange orchards, in Mediterranean climatic conditions. The main conclusions that can be pointed out are the following:

- DI strategies (i.e., RDI and PRD) did not alter soil fertility among treatments and compromise the nutrients uptake by plants;
- the sub-surface drip irrigation (SDI) and the control had similar behaviors, but SDI, allowing the reduction of soil evaporation losses, should be preferable to surface drip irrigation;

- the physiological response to water stress conditions did not show particular differences among the irrigation treatments, not inducing detrimental effects on crop production features;
- proline accumulation in orange leaves results were not related to water deficit conditions; rather, proline reached the highest values in the well-irrigated T1 and T2 treatments;
- ABA levels in leaves were rather constant in all the treatments, except in T4 (PRD) during September; this response might produce a late adaptive crop production response;
- prolonged drying cycles during alternate PRD exposed more roots to severe soil drying, thus increasing leaf ABA accumulation.

Case study 2

Frutticoltura - n. 1 – 2019

Risposta fisiologica di un agrumeto allo stress idrico controllato

6. Physiological response of a citrus grove to controlled water stress

Helena Pappalardo, Elisabetta Nicolosi, Daniela Vanella, Biagio Torrisi, Maria Allegra, Fiorella Stagno, **Daniela Saitta**, Simona Consoli, Giancarlo Roccuzzo, Filippo Ferlito

Introduction

Citrus growing, with an area of about one million hectares in the Mediterranean area, represents one of the most economically relevant fruit growing realities (Roccuzzo et al., 2015). To ensure the competitiveness of the sector, the agronomic inputs necessary for the optimal management of the citrus grove are huge.

Among these, the irrigation contribution is undoubtedly a significant factor for the sustainability of the citrus grove. The adoption of "Deficit Irrigation" (DI) or controlled water deficit strategies, increasingly known in recent years, is a useful choice to allow reduction of management costs and improvement of the agro-ecological value of crops, as well as for the maintenance and improvement of some quantitative and qualitative characteristics of productions.

Considering that the water resource in agriculture is increasingly limited due to the strong competition, for its use, with other sectors (industrial and tourism), in addition to the effects induced by the climate changes in progress, which increases in temperature, suboptimal distribution of rains and reduction of annual rainfall, the rational management of irrigation is a particularly important crop aspect. According to the Intergovernmental Panel on Climate Change (IPCC, 2014), the concentration of atmospheric CO_2 has increased, in less than 300 years, by 40%, and in the near future it will continue to grow with the same trend (Stich et al., 2008).

Therefore, considering that the availability of water is the main factor for the assimilation of CO_2 during photosynthesis (Lal et al., 2011), DI strategies become a useful tool to increase the "water use efficiency" of citrus groves and, in in particular, to obtain a positive balance between water saving and plant production.

The orange is a species with a particularly long productive vegetative cycle and an irrigation season. During the summer, if the soil moisture is limited and the roots are unable to guarantee the water supply, the evapotranspiration rate (ET) decreases and, consequently, growth decreases (Hsiao, 1973; Fereres and Soriano, 2007). Numerous researches have shown that the effectiveness of DI strategies depends on the phenological phase in which the stress is applied and on environmental factors (Consoli et al., 2014). In particular, the characteristics of the soil-plant-atmosphere (SPA) continuum (hydraulic characteristics of the soil, air temperature, evapotranspiration rates) influence the opening and closing of stomata and, therefore, the assimilation of CO_2 and its levels. plant photosynthesis (Ribeiro et al. 2009; Consoli et al., 2017). Increasingly accurate estimates of evapotranspiration needs allow to optimize DI strategies. Among the numerous techniques examined, micrometeorological methods, such as the "Eddy Covariance" (EC), quantify the current ET (ET_a) starting from the direct measurement of the latent heat flow (LE) above the foliage.

This requires constant monitoring of water conditions soil and air temperature, as well as the monitoring of mass flows in the SPA continuum of the physiological state of plants (photosynthesis, transpiration and canopy temperature) during the irrigation season.

The objective of the study was to evaluate the eco-physiological response of a citrus grove subjected to controlled water stress conditions following the adoption of DI strategies, in a production reality, such as the citrus fruit of eastern Sicily, still too tied to massive water supplies, in order to offer protocols of sustainable irrigation management.

Materials and methods

Site, irrigation strategies and water consumption estimation

The study is conducted at the Palazzelli experimental farm

(37 ° 17 'N, 14 ° 50' E; Lentini, Sr), of the CREA Centro di Ricerca Olivicoltura, Frutticoltura e Agrumicoltura di Acireale (Ct), in a plot of orange (Citrus sinensis (L.) Osbeck) aged 12 years, implanted with the cultivar Tarocco clone Sciara C1882, grafted on the rootstock Citrange Carrizo [Poncirus trifoliata (L.) Raf. X C. sinensis (L.) Osbeck] with a planting spacing of 6 x 4 m. The soil is sandy-loam and with good general fertility (Consoli et al., 2014, 2017). The climate is typically Mediterranean semi-arid, with annual rainfall sparsely distributed in autumn and winter.

Since 2010, the following irrigation treatments have been tested in situ: control (C), in which 100% of the evapotranspiration is distributed cultivation (ETc), through a drip irrigation system superficial; “Sustained Deficit Irrigation” (SDI), in which 75% of ETc is administered, through a drip sub-irrigation system placed at a depth of 0.35 m; “Regulated Deficit Irrigation” (RDI), in which the water deficit is modulated according to the phenological phases of the plant (subsequent phase the physiological drop of the fruit), returning 50% of ETc from half July to the end of September through a superficial drip micro-irrigation system; "Partial Root-zone Drying" (PRD), whose deficit water is equal to 50% of the ETC and irrigation is distributed through the alternate activation of two 2 water pipes placed laterally with respect to the rows.

The programming of the irrigation volumes applied to the various treatments, in the summer period (June - September 2018), is determined by resorting to the estimation of crop water needs (ET_c) through the "mean crop coefficient" (K_c) approach (Allen et al., 1998, 2006). This provides for the estimate of the reference ET (ET_0) calculated using the climatic data from the weather station present on site through the Penman-Monteith formulation. Once the ET_0 values are known, the ETC estimate is carried out, on a weekly basis, by applying specific reduction coefficients linked to the crop type ($K_c = 0.7$) and the crown / soil coverage ratio with respect to the planting layout ($Kl = 0.7$) and taking into account any meteoric contributions during the irrigation period. Control of distributed irrigation volumes is carried out by reading volumetric meters placed on the supply lines.

Since 2016, the experimental field has been equipped with an "Eddy Covariance" (EC) system for monitoring the various components of the surface energy balance in the SPA continuum and the ETa flows. The EC system consists of a three-dimensional sonic anemometer (CSAT3-3D, Campbell Scientific Inc.) and an open circuit gas analyzer (Li-7500, Li-cor Biosciences Inc.) for the high frequency measurement of the three components of the wind speed and concentrations of water vapor and CO_2 (**Figure 1**). The raw data acquisition frequency is 10 Hz. Data processing and EC measurement processing is performed in accordance with Euroflux standard protocols (Aubinet et al., 2000).

Figure 1. Micrometeorological tower installed at the experimental site.

Eco-physiological monitoring of plants

The eco-physiological response relating to the DI regimes applied is assessed on 16 index plants (4 plants per treatment), through specific surveys carried out every two weeks in the irrigation period (June-September 2018). In particular, it was monitored the temperature of the canopy, transpiration and leaf photosynthesis.

The canopy temperature was measured at noon using a laser thermometer on 4 leaves exposed to the sun in the direction of the cardinal points (N-S-W-E). The canopy temperature values in the 4 directions were averaged per plant.

Leaf photosynthesis and transpiration were detected by means of a portable gas exchange measurement system (LI-Cor 6400, Li-Cor Inc., Lincoln, NE, USA) (**Figure 2**) consisting of a standard leaf chamber having an area of 6 cm^2. Measurements were made on 3 leaves per plant.

The instrument has been calibrated to maintain the balance between the CO_2 inside and outside the chamber in the absence of measurements. The duration of the measurement of photosynthetic yield and leaf transpiration was approximately 2–3 minutes. The collected data was subjected to statistical analysis (Anova and Tukey's HSD test).

Figure 2. Monitoring of eco-physiological parameters.

Application of "proximal sensing" techniques

During the 2018 irrigation season an application of "proximal sensing" (acquisition of images in the visible and thermal infrared with portable instruments) was carried out to monitor the water status of the control and treatment PRD deficit.

The visible images were acquired using a grayscale “global shutter” camera (oCam-1MGN-U), with 111 ° x65 ° optics and a spatial resolution of 1280 x 960 pixels. Thermal images were acquired using a camera thermal (Optris PI 160), with optics of 72 ° x52 ° and resolution of 160 x 120 pixels, with an accuracy of ± 2 ° C.

The application of "proximal sensing" techniques made it possible to analyze an area of extension of approximately 900 m2 including 18 plants respectively in the two treatments.

Prior to the acquisition, ground benchmarks were identified using the GPS system (Leica GS15) with "real time" correction by RTK Italpos (reference ellipsoid WGS 1984 and reported ellipsoidal height), to be used as a reference for the subsequent georeferencing of the images. been acquired at a distance such as to obtain a “ground sampling distance” (GSD) of 33 and 113 mm / pixel respectively in the visible and in the thermal. The individual frames were afterwards georeferenced and mosaics through photogrammetric procedures. The thermal characteristics of individual plants in the study belonging to the 2 irrigation treatments were extracted from the thermal image in the GIS environment through procedures manual typing of the foliage of the plants on the image in visible and extraction of zonal statistics from the thermal image.

Results and discussion

Deficit irrigation strategies and water consumption.

The applied DI strategies made it possible to obtain water savings of approximately 21, 32 and 52% in the SDI, RDI and PRD deficient treatments compared to the control (312.4 mm). The flows of ET_0, ET_c and ET_a recorded in the period under study (June-September 2018) are equal to 646, 317 and 234 mm, respectively.

Eco-physiological monitoring of the crops under study

The applied DI strategies did not show conditions of prolonged stress to the plants. At the beginning of the irrigation season, the control showed foliage temperatures lower than the PRD treatment, but higher than the other treatments (SDI and RDI). Subsequently, the temperatures of all plants reached their highest peak from mid-July to the end of the month (average value 33 ° C), while starting from mid-August,

thanks also to significant and repeated rainy events, there was a constant cooling up to the end of September (average value 26.7 ° C; Fig. 3).

At the end of August compared to the control, the SDI and RDI treatments showed a decrease in temperature (3.5 and 2.8%), while the PRD one showed an increase (1.2%) thus restoring between treatments, for a limited period, the conditions observed at the beginning of the irrigation season. In an inversely proportional way with respect to what reported for the temperature of the foliage, the photosynthetic rate remained at values lower than 4 mol CO_2 m^{-2} s^{-1} from the start of monitoring until mid-August, reaching the highest peak (7, 62 mol CO_2 m^{-2} s^{-1}) at the end of this month (Fig. 4). As expected, the optimum for photosynthesis was recorded with temperatures between 25 and 30 ° C as reported in the literature (Guo et al. 2006).

Leaf transpiration was significantly affected by treatments (data not shown). This reaches its highest peak in mid-July with an average value of 1.24 µmol H2O m^{-2} s^{-1} and its lowest level in late July with an average value of 0.12 µmol H2O m^{-2} s^{-1} (Fig. 5), moreover at the time when the leaf temperature was higher. In particular, on average, there was a reduction of 34% in PRD compared to the control, of 32 and 18% in SDI and RDI respectively.

Compared to the control, perspiration in the last 2 dates significantly decreased in PRD (60.3% and 45.9%).

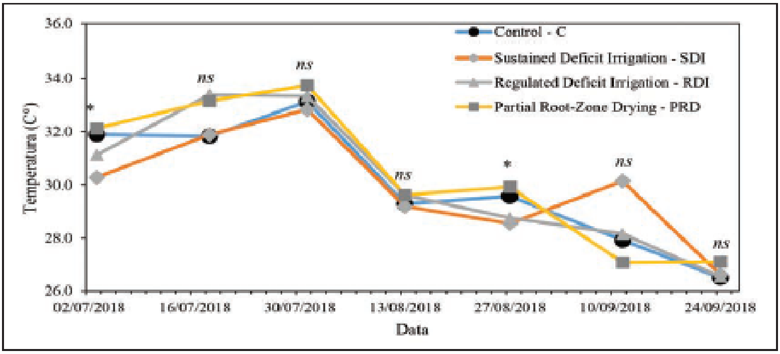

Figure 3. Crown temperatures recorded during the irrigation season (*: significant differences $p \leq 0.05$; ns: not significant).

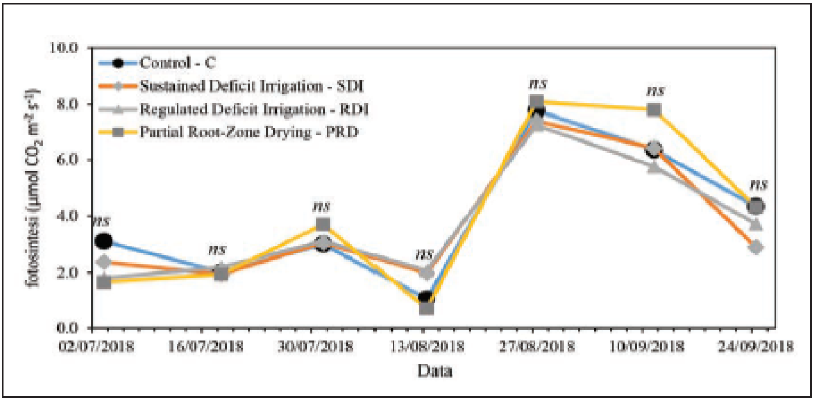

Figure 4. Levels of photosynthesis recorded during the irrigation season. (*: significant differences $p \leq 0.05$; ns: not significant).

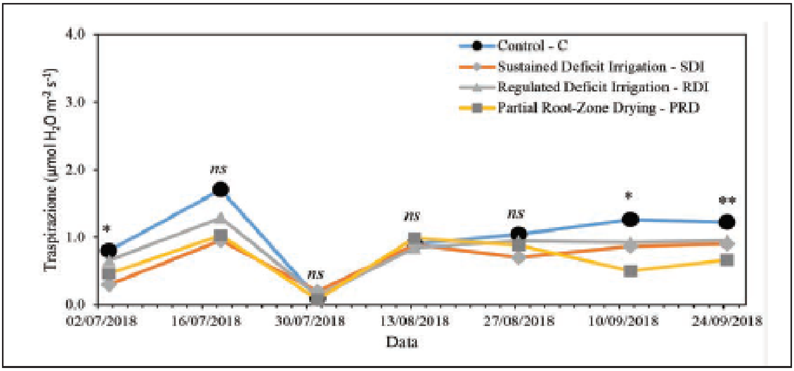

Figure 5. Transpiration rates recorded during the irrigation season. (*: significant differences $p \leq 0.05$; **: significant differences $p \leq 0.001$; ns: not significant).

Application of "proximal sensing" techniques

The analysis of the thermal maps obtained through the "proximal sensing" techniques allowed to highlight, as expected, a higher thermal delta in the PRD treatment. **Figure 6** shows the information on the temperatures detected through the thermal image referring to the areas occupied by the canopy of the control and PRD treatment plants. The temperature tree crown average in C and PRD is respectively 35.4 (± 0.24) and 36.1 ° C (± 0.18), compared to an air temperature of 33.3 ° C at time of monitoring. The thermal delta (ΔT) in C and PRD is equal to 2.1 (± 0.24) and 2.8 ° C (± 0.18) respectively.

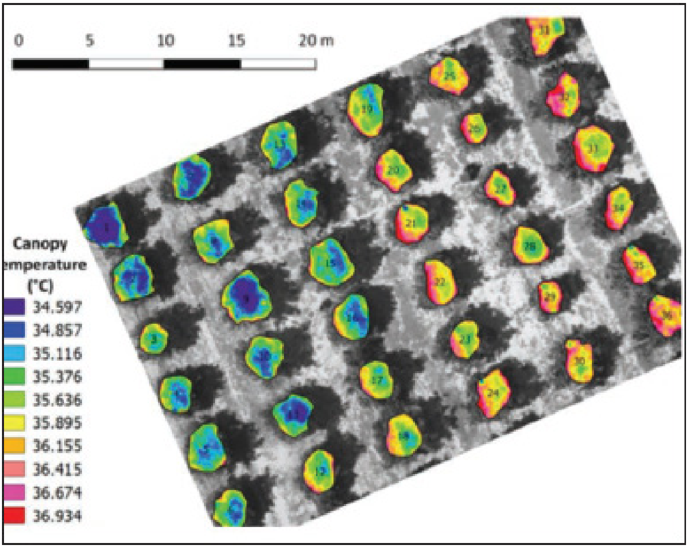

Figure 6. Crown temperatures of Control plants (1–18) and of "Partial Root-zone Drying" (19–36) obtained through "proximal sensing" techniques.

Conclusions

In conclusion, the study still in progress shows that when the climatic conditions are less limiting, the effects due to the different insufficient irrigation strategies become more evident. Among the measured parameters the photosynthetic rate of the plant appears being the least subject to water stress and high temperatures, therefore the phases of growth of the fruit by cellular extension that require a good water status of the plant, can be effectively supported.

In general, the strategies adopted guarantee positive feedback for the reduction of the volumes of water to be administered.

Case study 3

Journal of Irrigation and Drainage Engineering, 2020, 146(7): 05020002

7. Comparison of orange orchard evapotranspiration by eddy covariance, sap flow and fao-56 methods under different irrigation strategies

Daniela Saitta, Daniela Vanella, Juan Miguel Ramírez-Cuesta, Giuseppe Longo-Minnolo, Filippo Ferlito, and Simona Consoli

Abstract

The study evaluates the accuracy of measured and estimated crop evapotranspiration fluxes (ET_c) on Citrus in a semiarid Mediterranean climate (Sicily, Italy). Specifically, ET_c rates derived from in situ techniques [eddy covariance (EC) and sap flow heat pulse velocity (HPV)] and modelling approaches [Food and Agricultural Organization of the United States (FAO) Irrigation and Drainage Paper No. 56 (FAO-56) single and dual crop coefficient (K_c)] were compared under deficit irrigation scenarios. Results of the comparison showed that the single and dual K_c approaches provided similar ET_c estimates (292 and 324 mm), even if these approaches overestimated ETc measured by EC (ET_{EC}) (17% and 30% respectively). HPV was able to show transpiration (T) reductions caused by deficit irrigation strategies when compared with T under full irrigation condition (ranging from 70% to 82%). Overall, the assessed methodologies were able to capture ET_c trends, but the selection of the most appropriate one will depend on the specific crop and study site characteristics.

Author keywords: Deficit irrigation; Crop coefficients; Transpiration; Micrometeorology; Citrus orchards.

Introduction

Citrus orchards, widely diffused in Mediterranean regions covering around 1,000,000 ha, have a major role in the economy of these areas (FAO, 2003). With the peculiarity of its semi-arid climate, Sicily (Southern Italy) has an excellent potential for orange production. This region is characterized by rainfall scarcity conditions and therefore *Citrus* trees need of irrigation to guarantee profitable production. Limited water availability is the results of concomitant factors such as periodic drought, increasing competition for water resources, irrigation infrastructure failures, and climate change scenarios. This latter, in particular, implies air temperature and evapotranspiration (ET) increases and precipitation reduction (Faurés et al., 2013). Such a scenario needs more efficient uses of the limited water resources, which imply the accurate determination of water requirements of *Citrus* orchards (i.e. ET) and the adoption of sustainable irrigation strategies (i.e. controlled deficit irrigation) (Capra et al., 2008; Consoli et al., 2016; Consoli and Papa 2013; Consoli and Vanella 2014; Er-Raki et al., 2009; Maestre-Valero et al., 2017; Motisi et al., 2012).

The easiest and cheaper approach to estimate ET is the FAO-56 method (Allen et al., 1998, 2006). FAO-56 includes a single crop coefficient (K_c) approach, and a more complex dual K_c approach (basal crop coefficient, K_{cb}; and evaporation coefficient, K_e) that splits the ET in crop transpiration (T) and soil evaporation (E) (Allen et al. 1998). Both approaches account for climatic variables in the reference ET (ET_0) and for crop type and its characteristics (included in K_c and K_{cb}), whereas soil texture and hydraulic characteristics are included in K_e. Despite being the most widely used approach up to date, the methodology requires crop-specific coefficients and field validation in order to offer reliable estimates.

As reported in numerous studies, the adoption of the dual K_c approach results more appropriate in case of application of deficit irrigation scenarios, such as the partial zoot-zone drying (PRD), and the adoption of drip irrigation technologies (Anderson et al., 2017; Ding et al., 2013; Ferreira et al., 2012; O'Connell and Goodwin 2007; Odhiambo and Irmak, 2012; Paco et al., 2012). Different studies have analysed the results provided by the FAO-56 approach (i.e. single or dual) and those derived from methodologies based on the Surface Energy Balance, sap flow, Eddy Covariance (EC), Surface Renewal, and Bowen Ratio (Casa et al., 2000; Consoli et al., 2006; Consoli and Papa 2013; Er-Raki et al., 2009; Maestre-Valero et al., 2017; Motisi et al., 2012; Paco et al., 2006; Vanella and Consoli 2018). Among the different methodologies, EC is considered as the most reliable and accurate method for measuring ET of tree crops, if, of course, measurement requirements are met (Baldocchi et al., 1988; Snyder et al., 2000). Nevertheless, this technique does not distinguish between T and E, being necessary to use other methods for assessing each component separately. Thus, T can be effectively evaluated using sap flow methods (Agam et al., 2012; Heilman and Ham 1990; Thompson et al., 1997; Rafi et al., 2019). A variety of methods are available to calculate water mass flow within stem using heat as a tracer (Heat Balance Method, Vieweg and Ziegler 1960; and Heat Pulse Velocity, HPV, Swanson and Whitfield 1981; Green 1998; among others). Measurements can be carried out in herbaceous and woody plants and in any conductive organ, including roots. The up-scaling of T fluxes to field scale is generally carried out via crop specific characteristics, such as leaf area index (LAI) (Motisi et al., 2012; Oishi et al., 2008; Soegaard and Boegh 1995).

When direct measurements are not available, separate evaluations of E and T fluxes can also be obtained by using agro-hydrological models, like, for example, the surface-vegetation-atmosphere-transfer (SVAT) model, which determines the temporal dynamics of the two separated fluxes using physically-based schematizations (Crow et al., 2008; Rallo et al., 2012).

In literature it is possible to find numerous studies on the comparison of different methods for estimating ET_c under full irrigation conditions (Poblete-Echeverría et al., 2013; Raki et al., 2009; Tian et al., 2016), but non consensus has been achieved about the effectiveness of these methods under deficient irrigation conditions. Therefore, one of the main novelties of this study is to bring to light new information about the performance of different methods for ET_c determination under deficit irrigation strategies in heterogeneous crops. More specifically, in this study we focused on the comparison between single and dual K_c FAO-56 approaches, EC and sap flow techniques to obtain reliable crop ET (ET_c) fluxes of orange orchards in Eastern Sicily (Italy). The objectives were (1) to evaluate whether the integration among different methodologies is appropriate for accurately estimating the ET_c over an irrigated orchard in the semi-arid climatic conditions and (2) to identify the impact of the applied deficit irrigation strategies on the performance of the proposed methodologies.

Materials and methods

Site description

The study site is a 1.0 ha experimental orange orchard in Eastern Sicily, Italy (37° 20' N, 14° 53' E; Fig. 1). Orange trees cv Tarocco Sciara, grafted on Carrizo citrange, [*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck], were planted with a spacing of 6.0 m (between-row) x 4.0 m (within-row). The site has a semi-arid Mediterranean climate with irregular rainfall (i.e. yearly average of about 500 mm) an annual ET_0 of about 1,400 mm (Capra et al., 2013).

The trial was a randomized block design, which includes four irrigation treatments, replicated three times (Consoli et al. 2014): (i) a control, irrigated at full (T1, 100% ET_c), adopting surface drip irrigation; (ii) sustained deficit irrigation (SDI, T2) irrigated to replenish 75% ET_c, adopting subsurface drip irrigation; (iii) regulated deficit irrigation (RDI, T3) irrigated at 100% ET_c except during the fruit growth (50% ET_c); and (iv) partial root-zone drying treatment (PRD, T4) irrigated at 50% ET_c where wet and dry parts of the root-zone were alternated at 7 days interval. Each treatment consists of 24 plants, distributed in three rows of eight trees each (**Figure 1**). Two irrigation seasons were considered in the study (2018-2019). Irrigation was applied early in the morning three times per week from mid June (DOY 169 and 168 for 2018 and 2019, respectively) to end September (DOY 273 and 280 for 2018 and 2019, respectively).

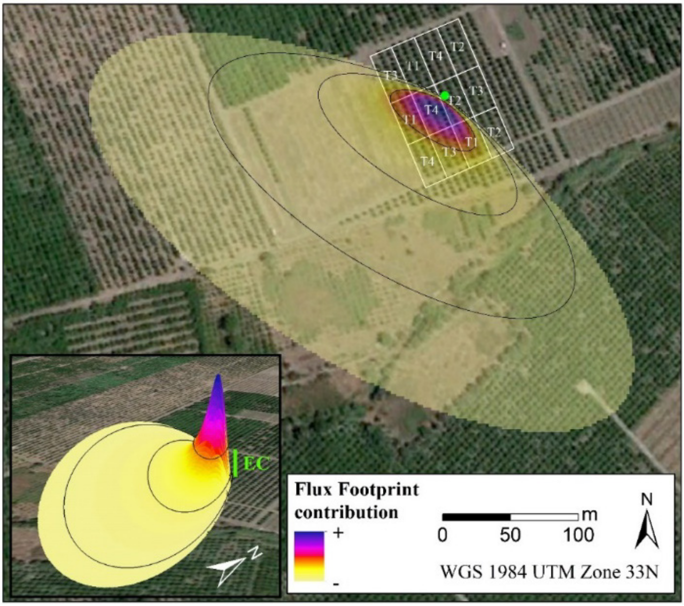

Figure 1. Layout of the deficit irrigation experimental site (T1–T4) and EC flux footprint: (a) the point marks the EC tower, around which the lines indicate the quartile contour lines of flux EC footprint contributions averaged over 2018; and (b) corresponding three-dimensional (3D) flux footprint contribution.

Water derived for irrigation was characterized by a medium salinity (electrical conductivity of about 2.0 dS m^{-1}) and pH of about 7.0. Soil texture and hydraulic characteristics were investigated within the profile 0.05-0.25 m. From laboratory determinations soil samples resulted fairly uniform with a sandy-loam texture. The soil field capacity and permanent wilting point were respectively of 28% and 14% (Aiello et al., 2014; D'Emilio et al., 2018).

Evapotranspiration estimates by the FAO-56 approaches

Reference ET (ET_0, mm d^{-1}) was estimated using the Penman-Monteith formula (Allen et al., 1998; Allen et al., 2006) (Eq. 1).

$$ET_0 = \frac{0.408\Delta\left(R_n - G\right) + \gamma \frac{900}{T_a + 273} u_2\left(e_s - e_a\right)}{\Delta + \gamma\left(1 + 0.34u_2\right)} \qquad (1)$$

Meteorological data (i.e. solar radiation, R_s, W m^{-2}; air temperature, T_a, °C; relative humidity, RH, %; wind speed, u_2, m s^{-1}; and rainfall, mm) were measured hourly from an automatic weather station nearby the experimental field.

In Eq. 1, Δ is the slope of the saturation vapour pressure at the T_a (kPa $^oC^{-1}$); R_n is the net radiation (MJ m^{-2} d^{-1}); G is the soil heat flow (MJ m^{-2} d^{-1}); γ is the psychrometric constant (kPa $^oC^{-1}$); e_s-e_a is the vapour pressure deficit (kPa), and u_2 refers to wind speed at 2 m height (m s^{-1}).

ET_c was derived using the single K_c FAO-56 approach ($ET_{c,\,Kc}$) by multiplying daily ET_0 by the seasonal crop coefficient for orange orchard (K_c) (i.e. 0.7 as observed by Consoli et al. 2006; Consoli and Papa 2013). Irrigation rates were then adjusted according to rainfall and to a localization factor depending on the canopy area (K_l) (i.e. 0.7).

The dual K_c FAO-56 approach determines ET_c ($ET_{c,\ dual\ Kc}$) by separating K_c in two coefficients accounting for T (K_{cb}) and E (K_e), as follows:

$$ET_{c,dual\ Kc} = (K_{cb} + K_e) \cdot ET_0 \tag{2}$$

In Eq. (2), K_{cb} is the ratio between ET_c and ET_0 in conditions of soil surface dry and soil water content adequate to maintain full T (Allen et al., 1998). K_e depends on the available water at the topsoil and its requires a daily soil water balance (SWB) in order to determine the cumulative E, or depletion from the wet condition, as follows:

$$K_e = K_r\left(K_{c,max} - K_{cb}\right) \tag{3}$$

$K_{c,max}$ is the maximum K_c value after rain or irrigation while K_r represents the reduction on E depending on the evaporation process, as follows:

$$K_r = \frac{TEW - D_{e,i}}{TEW - REW} \tag{4}$$

where TEW = 1000 ($\theta_{FC} - 0.5\ \theta_{WP}$) Z_e, is the total evaporable water (i.e., maximum depth of water that can be evaporated from the soil surface layer), with θ_{FC} and θ_{WP} measured at the study site (28 and 14%, respectively) and Z_e equals to 0.1, as reported in Allen et al. (1998). REW is the readily evaporable water (fixed at 10 mm for the study site soil, i.e. sandy loam; Allen et al., 1998); and $D_{e,i}$ is the cumulative depth of E from the topsoil (mm) at the end of the ith day and it is solved, as follows:

$$D_{e,i} = D_{e,i-1} - (P_i - RO_i) - \frac{I_i}{f_w} + \frac{E_i}{f_{ew}} + T_{ew,i} + DP_{e,i} \tag{5}$$

where $D_{e,i-1}$ is the cumulative depth of E after a total wetting from the exposed and wetted fraction of the topsoil at the end of day i-1 (mm); P_i is rainfall (mm); RO_i is runoff from the soil surface (mm); I_i is irrigation which infiltrates the soil (mm); E_i is evaporation (i.e., $E_i = K_e\ ET_0$) (mm); $T_{ew,i}$ is depth of T from the exposed and wetted fraction of the soil surface (mm); $DP_{e,i}$ is deep percolation loss from the topsoil layer if soil water content exceeds θ_{FC} (mm); f_w is fraction of soil surface wetted by irrigation (0.1 based on the wet bulb area as observed in Vanella et al. 2019); f_{ew} is exposed and wetted soil fraction, computed as the lowest value between the average exposed soil fraction not covered and f_w.

Eddy Covariance Evapotranspiration fluxes

In 2016, a 7 m-tall EC tower (Fig. 1) was installed at the study site (about two times the canopy height). EC flux footprint (**Figure 1**) was calculated using a cross-sectional approach, which considers the atmospheric stability, measurement height, surface roughness length (Hsieh et al., 2000) and the lateral flux dispersion (Eckman 1994; Schmid 1994; Detto et al., 2006).

The EC tower consists of one three dimensional sonic anemometer (CSAT3-3D, Campbell Scientific Inc., Logan, UT, USA) and one infrared open-path gas analyzer (Li-7500, Li-cor Biosciences Inc., Lincoln, NE, USA) to measure high frequency (10 Hz) wind components and the H_2O and CO_2 concentrations. Low frequency energy fluxes (30-min), R_n (W m^{-2}) and G (W m^{-2}) were measured using one net radiometer placed 7 m above the ground (CNR-1 Kipp & Zonen, Delft, The Netherlands) and three self-calibrated soil heat flux plates (HFP01SC, Hukseflux, Delft, The Netherlands) placed in the exposed, half-exposed and shadowed soil, at a depth of about 0.05 m. High and low frequency data were collected in a CR1000 logger (Campbell Scientific Inc., Logan, UT, USA).

The standard EUROFLUX corrections (Aubinet et al., 2000) were adopted for EC measurements and data processing. The sensible heat flux (H, W m^{-2}) was computed as:

$$H = \rho \cdot c_p \cdot \sigma_{wT} \tag{6}$$

with ρ (g m^{-3}) the air density, c_p (J g^{-1} K^{-1}) the air specific heat capacity at constant pressure, and σ_{wT} (m s^{-1} K) the covariance between the vertical wind speed and air temperature.
The latent heat flux (λET, W m^{-2}), was:

$$\lambda\mathrm{ET} = \lambda \cdot \sigma_{\mathrm{wq}} \tag{7}$$

with λ (J g^{-1}) the latent heat of vaporization and σ_{wq} (g m^{-2} s^{-1}) the covariance between the vertical wind speed and water vapour density.
The surface energy balance closure ratio (CR) is computed as:

$$CR = \frac{(H + \lambda ET)}{(R_n - G)} \tag{8}$$

CR allows to determine how well the turbulent fluxes of heat and water vapour account for the available energy (R_n-G) (Prueger et al., 2005).
Half-hourly fluxes were daily aggregated and λET data were then transformed to equivalent depth of actual ET (ET_{EC}, mm d^{-1}).
Using the ET_{EC} measurements, the corresponding K_c ($K_{c,EC}$) was estimated as in the follows:

$$\mathrm{K}_{\mathrm{c,EC}} = \frac{\mathrm{ET}_{EC}}{\mathrm{ET}_0} \tag{9}$$

<u>*Sap flow estimates*</u>
Water consumption was continuously measured in two trees per irrigation treatment using the HPV sap flow method

(Swanson and Whitfield 1981). The HPV technique is based on measurements of temperature variations (ΔT), caused by a heat pulse of short duration (1-2 s), in two probes installed asymmetrically into the tree trunk. Each probe consists of one 4 cm sensor with two thermocouples embedded (Tranzflo NZ Ltd., Palmerston North, NZ. It was wired to a data-logger (CR1000, Campbell Sci., Logan, UT, USA) for heat-pulse measurement at 30-minute sampling intervals. Sap Data were runned according to Green et al. (2003) and sap flow velocity was integrated over the sapwood area for estimating transpiration (T) fluxes. For this purpose, fractions of wood (F_M=0.48) and water (F_L=0.33) in the sapwood were evaluated on the trees where sap flow probes were posed. F_M and F_L were measured in wood samples (5 mm diameter, 40 mm length) taken with an increment borer in the proximity of the probe sets (Consoli et al., 2017). This requires the input of fresh weight, oven-dried weight, and immersed weight (Si et al., 2009).

Results

Irrigation rates supplied during 2018 at the different treatments were 312 mm (T1), 248 mm (T2), 211 (T3) and 150 mm (T4). The percentages of water saving obtained respect to the fully irrigated control (T1) were about 20% for SDI (T2), 32% for RDI (T3) and 52% for PRD (T4). For 2019 irrigation rates supplied at the different treatments were 302 mm (T1), 250 mm (T2), 225 (T3) and 142 mm (T4), with savings respect to the control (T1) of 17% for SDI (T2), 25% for RDI (T3) and 53% for PRD (T4). The evolution of ET_0 (mm d^{-1}), which is critical in the determination of crop water requirements, is illustrated in **Figure 2.**

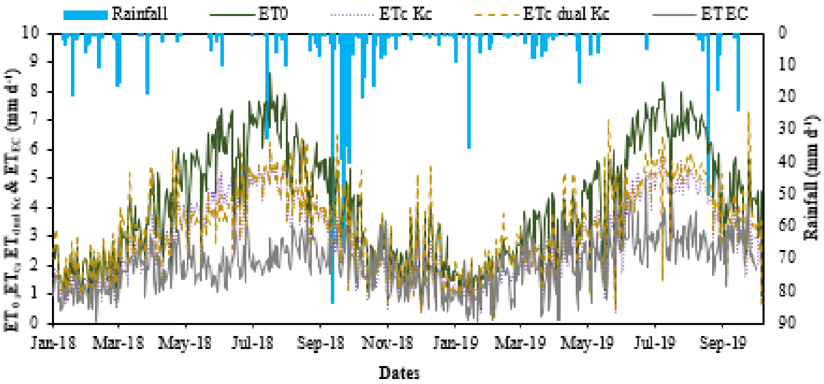

Figure 2. Daily ET0, $ET_{c,\ Kc}$, $ET_{c,\ dualKc}$, and ET_{EC} during the period 2018–2019 at the experimental site.

At the orchard level, the temporal ET_0 pattern is typical of semi-arid conditions, characterized by high evaporative demand, with a cumulative ET_0 of about 2,580 mm for the period January 1, 2018 (DOY 1, 2018) to October 7, 2019 (DOY 280, 2019). Within the considered period, the lowest and highest ET_0 values occurred during winter 2019 (0.1 mm d^{-1}) and summer 2018 (8.6 mm d^{-1}), respectively. The cumulative rainfall for the same period was 696 mm. The cumulative $ET_{c,Kc}$ obtained for the reference period using the single K_c approach was 1,264 mm, while $ET_{c,dual\ Kc}$ derived from the dual-K_c method was about 1,485 mm. The rainfall regime occurred at the study site indicates the necessity to adopt irrigation strategies for optimizing water allocation, while maintaining profitable yield.

Surface energy balance CR was checked by comparing the λET and H measured by EC technique with R_n and G [**Figs. 3**(**a**)(**b**)]. In our study, the lack of closure, which indicates the consistence and strength of EC data, was about 18% and 19%

[**Figs. 3**(**a**)(**b**)] for 2018 and 2019, respectively. Therefore, the present energy balance CR was considered to provide evidence for the validity of the results on λET.

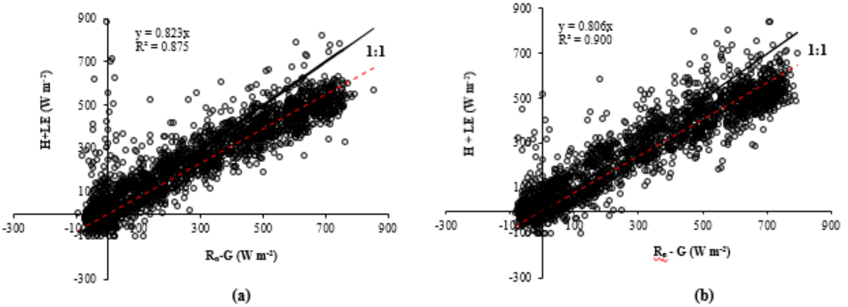

Figure 3. Energy balance closure with half-hourly fluxes measured during June–September (a) 2018; and (b) 2019 at the experimental site.

According to the footprint analysis, almost 40% of the fluxes sensed by the EC system come from the experimental area (**Figure 1**). The analysis also showed that EC fluxes measured at 7 m height were mainly affected (75%) by fluxes coming from an upwind area (NE-SW wind direction) at a distance of about 150 m from the tower, in part characterized by ground cover heterogeneity.

Measured ET$_{EC}$ (mm d^{-1}) for the considered period ranged from 0.1 to 5.0 mm d^{-1} (**Figure 2**), with a cumulative value of 1,281 mm. During the irrigation periods, mean daily ET$_{EC}$ values were 2.4 and 2.8 mm d^{-1} for 2018 and 2019, respectively; with a cumulated value of 250 mm (2018) and 303 mm (2019). For both the irrigation seasons, the mean daily ET rates retrieved by the single and dual-K$_c$ approaches were 2.8 and 3.1 mm d^{-1}. Cumulative rates of ET$_{c,Kc}$ and

$ET_{c,dual\ Kc}$ were 17 and 30% higher than ET_{EC} during the 2018 irrigation period (with values of 292 and 324 mm, respectively) and 4 and 17% higher during the 2019 irrigation season (with values of 316 and 356 mm, respectively).

The temporal pattern of the daily mean K_c for the two different irrigation seasons, obtained from the different approaches, are illustrated in **Figure 4**. For In 2018, the single K_c, $K_{c,dual}$, and $K_{c,EC}$ were 0.70, 0.79 and 0.45, respectively; whereas in 2019, these values were 0.70, 0.82 and 0.54, respectively. $K_{c,EC}$ values were 23-36% and 34-43% lower than the estimated values by the single and dual K_c approaches.

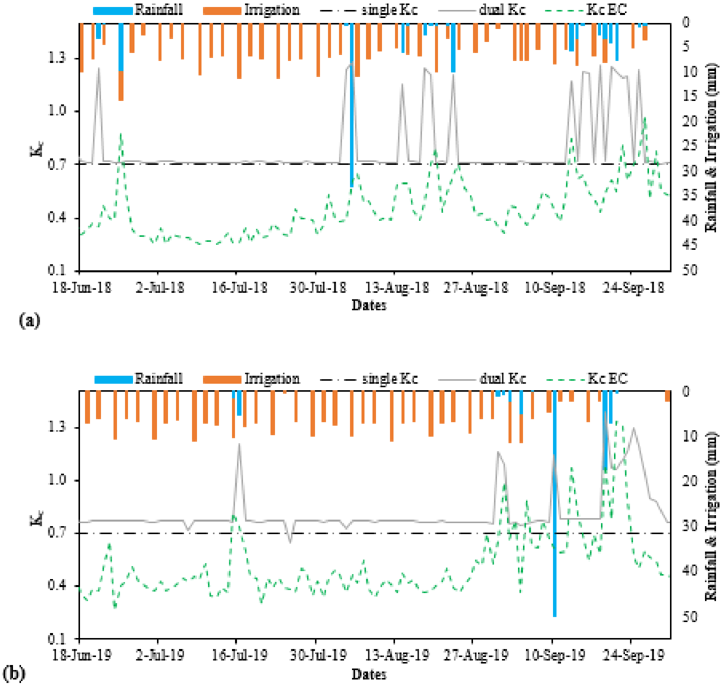

Figure 4. Daily Kc (K_c, $K_{c,dual}$, and $K_{c,EC}$), rainfall and irrigation during the irrigation season for (a) 2018; and (b) 2019 at the experimental site.

Figure 5 reports the daily T rates (mm d^{-1}) for the different irrigation treatments (T1-T4) by HPV, compared with ET_{EC} (mm d^{-1}) and T estimated by the dual K_c FAO-56 approach ($T_{dual,Kc}$). According to sap flow data, the fully irrigated treatment (T1) transpired a mean of 1.36 mm d^{-1} during late August (DOY 237) to the end of September (DOY 273) 2018; whereas T fluxes in the deficit irrigated treatments T2 (SDI), T3 (RDI) and T4 (PRD), were respectively of 1.11, 1.03 and 0.94 mm d^{-1}, about 82, 76 and 70% of T rates measured in

T1. In 2019, the full irrigation treatment (T1) transpired a mean of 1.40 mm d^{-1} during late August (DOY 237) to early October (DOY 280), whereas T fluxes in the deficit irrigated treatments T2 (SDI), T3 (RDI) and T4 (PRD), were 0.92, 0.80 and 1.01 mm d^{-1}, for T2, T3 and T4, respectively (about 66, 57 and 72% of T rates measured in T1, respectively). In addition, T fluxes in T1 were on average about 57 and 50% lower than ET_{EC}, in 2018 and 2019, respectively.

In 2018, daily mean T and E rates obtained by the dual-K_c approach were respectively of 1.93 (mean $K_{cb} = 0.60$) and 0.74 mm d^{-1} (mean $K_e = 0.24$), with a mean $ET_{c,dual,Kc}$ of 2.68 mm d^{-1}. In 2019, daily mean T and E rates obtained by the dual-K_c approach were respectively of 1.81 (mean $K_{cb} = 0.64$) and 0.64 mm d^{-1} (mean $K_e = 0.24$), with a mean $ET_{c,dual,Kc}$ of 2.44 mm d^{-1}. Results from the dual-K_c approach revealed that E accounts for about 20% and 36% of the measured ET_{EC} for 2018 and 2019, respectively (Consoli et al., 2013a; Consoli et al., 2013b; Consoli and Papa 2013).

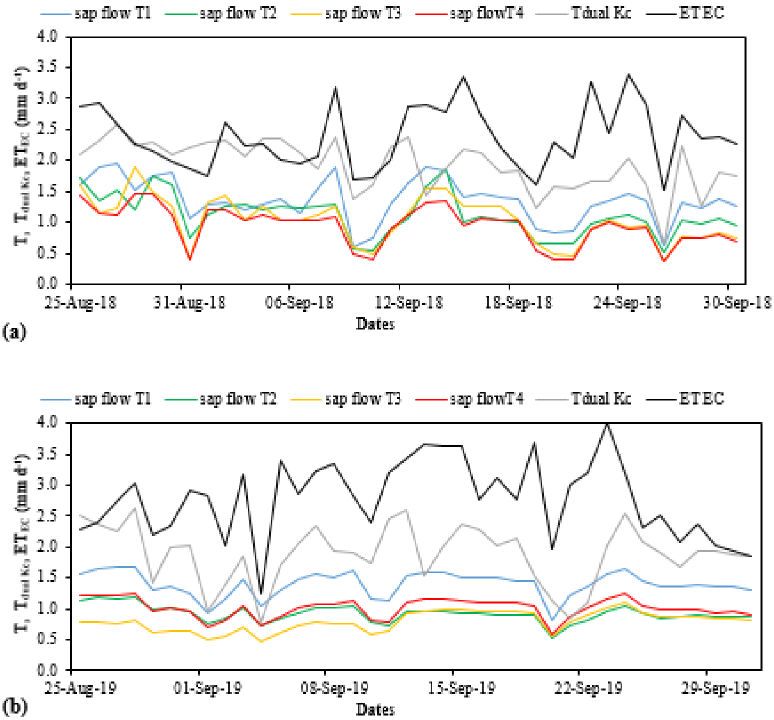

Figure 5. Daily T values (mm d^{-1}) of the different irrigation treatments by HPV sap flow (T1–T4), $T_{dual,Kc}$, and ET_{EC} for (a) 2018; and (b) 2019.

Discussion

During the irrigation seasons 2018-2019, the ET_c fluxes, obtained from the FAO-56 Kc approaches, and the EC showed different patterns as a result of the different ways in which K_c is estimated.

In the single K_c approach, crop coefficient followed a constant pattern, even if irrigation or rainfall occur, since this methodology only considers tabulated values as a function of

the crop stages (Allen et al., 1998). On the other hand, $K_{c,dual}$ and $K_{c,EC}$ showed the same behaviour when irrigation and/or rainfall occurred as an increase in the soil evaporative fraction (K_e component). However, when both irrigation and rainfall occurred the same day, $K_{c,dual}$ increases showed less amplitude than $K_{c,EC}$ (e.g. DOYs 176, 257, 263, in Fig. 4). These differences are related to f_w parametrization (Eq. 5). In fact, the dual K_c approach assumes that, when the soil surface is wetted by both irrigation and rain, f_w corresponds to the tabulated value, function of the adopted irrigation system (Allen et al., 1998). Additionally, the role of E component is particularly important for crops with incomplete vegetation fraction cover, as the study case, especially during the rainy season (Paço et al. 2014). Under these conditions, where irrigation or intense precipitation occurs (> 3-4 mm), the entire surface of the soil, including the area under the canopy, will be reached by it. It is precisely for this reason that it makes sense to assume f_w equal to 1 (Allen et al., 2006). Then, FAO 56 model represents a generalist procedure for ET_c estimation. Therefore, the estimations derived from this method must be considered with caution since this approach necessarily implies some generalizations that could not reflect the site specificities (Paco et al. 2006; Rafi et al. 2019)
In general, for both irrigation seasons, $K_{c,EC}$ values were lower than those obtained with single and dual K_c, which may be due to (i) an overestimation of the tabulated $K_{c,single}$ and $K_{c,dual}$ values, or to (ii) an underestimation in the ET_{EC}. Several authors have observed overestimations of FAO-56 tabulated values (Anderson et al., 2017; Consoli et al., 2006; Rosa et al., 2016; Vanella et al., 2019). For the dual K_c approach, these discrepancies are mainly caused by an

overestimation of tabulated K_{cb} and f_w, resulting in higher T and E estimates, respectively, the latter being more evident in the presence of sub-surface irrigation treatments (Cancela et al., 2015; Phogat et al., 2016).

On the other hand, several authors have highlighted the critical role of EC tower installation requirements for flux measurements (Burba and Anderson 2010; Giannico et al., 2018). A fundamental assumption in flux measurements based on aerodynamic principles (as EC) considers the area surrounding a tower as flat, large, and homogenous (Lee et al., 2004). However, even official flux networks, as FLUXNET, find difficulties for meeting these requirements, since such an ideal location rarely exists across global terrestrial surfaces (Giannico et al., 2018). Therefore, although EC technique is considered one of the most appropriate and accurate techniques for the direct measurement of ET_c (Novick et al. 2014; Perez-Priego et al., 2017; Zitouna-Chebbi 2017), it requires of large surfaces (especially for tall crops where the EC tower height is high), in order to minimize the influence of surrounding areas. The footprint analysis performed in this study indicated that the flux contribution area includes a heterogeneous region located outside the experimental site (**Figure 1**). Such heterogeneity, especially due to the presence of bare soil, resulted in a underestimation of $ET_{c,EC}$ and in a H overestimation, which explains the fact that at certain times $T_{dual,Kc}$ component was higher than $ET_{c,EC}$ (**Figure 5**). Additionally, the ET_c estimated from both single and dual K_c approaches assumes no stress conditions (e.g. stress coefficient, K_s, equals to 1). However, this assumption is partially met, since the deficit irrigation treatments (T2-T4) introduce some water stress into the agro-system, so it is expected that K_s is lower than 1, resulting in closer agreements among $ET_{c,\ single}$ and $ET_{c,dual}$ estimations and ET_{EC}.

The influence of the deficit irrigation treatments is also observed in T observations obtained from sap flow. Thus, T from T2-T4 were lower than T corresponding to the fully irrigated treatment (T1). These results are in agreement with those found by other authors, who also observed T reductions when deficit irrigation strategies were applied (Espadafor et al., 2017; López-López et al., 2018; Roccuzzo et al., 2014). Moreover, when comparing the T estimated by sap flow in T1 and $T_{dual,Kc}$, an overestimation of the latter is observed (**Figure 5**), supporting the hypothesis of an overestimation of the tabulated K_{cb} values (Abrisqueta et al., 2013). However, sap flow technique provides specific point-based measurements representative of single trees that are often linked to uncertainties related to the probe location (Burgess et al., 2001; Ren et al., 2017). Therefore, these punctual measurements need to be up-scaled to represent the field scale. In addition, the installation of a high number of probes in the field would also help to reduce errors caused by the malfunction of some probe or by the wound effect (Green et al., 2003; Marañón-Jiménez et al., 2017; Testi et al., 2009). However, its elevated costs limit the number of probes that may be installed in the field, reaching a compromise of using, as in this study, a pair or two pairs of them per experiment (Ballester et al., 2013; Lopez-Bernal et al., 2015). Another alternative is the use of approaches that directly provide spatially distributed ET_c estimations, as those based on remote sensing techniques (Allen et al., 2007; Bastiaannssen et al., 1998; Ramírez-Cuesta et al., 2019).

Conclusion

Different methods for estimating ET_c were compared, based on the single and dual K_c FAO-56 approaches and *in situ* observations (EC and sap flow techniques), in an orange orchard under deficit irrigation strategies during the irrigation seasons (2018-2019). The main findings of this study are summarized, as follows:

- the single and dual K_c approaches provided similar ET_c estimates, even if these approaches tended to overestimate ET_c respect to ET_{EC};
- EC measurements were affected by local specificity due to heterogeneity of the flux contribution area characterized by the deficit irrigation treatments and the surrounding bare soil fields;
- the partitioning between T and E in the dual K_c approach was in good agreement with T from sap flow and E determined as the difference between ET_{EC} and T from sap flow;
- the effects of water deficit applied at the different irrigation treatments were well-detected by the sap flow method at tree level and also influenced the performance of the different approaches (FAO-56 and EC) by reducing the ET_c rates at field scale;
- the selection of the most appropriate method for ET_c determination will depend on the crop specificities and the study site characteristics.

Case study 4

Agricultural Water Management, 2021, Vol. 247, 106734

8. Adaptation of citrus orchards to deficit irrigation strategies

Daniela Saitta, Simona Consoli, Filippo Ferlito, Biagio Torrisi, Maria Allegra, Giuseppe Longo-Minnolo, Juan Miguel Ramírez-Cuesta, Daniela Vanella

Abstract

In this study, the adaptation characteristics of orange trees, related to the application over a decade of deficit irrigation (DI) strategies, have been explored. To this purpose, the analysis of a minimal dataset composed of physiological information (stem water potential - Ψ_{stem} and sap flow - SF measurements), yield (fruits number and weight) and qualitative parameters (titratable acidity, TA; and total soluble solids, TSS) was performed with reference to the last irrigation seasons (i.e. 2018-19). The applied irrigation treatments were the following: sustained deficit irrigation (SDI); regulated deficit irrigation (RDI); partial root-zone drying (PRD), each distributing a water deficit of about 19%, 29% and 52%, respectively, compared to the control treatment (FI) supplying the full irrigation level (100% ETc). In general, higher water use efficiencies (WUE) have been obtained in DI treatments, which guarantee greater water savings (up to 50%), without affecting yield and quality characteristics. In particular, the most stressed treatment (PRD), while reaching the lowest Ψ_{stem} values (-1.8 - -2.0 MPa), as also shown by SF *versus* Ψ_{stem} clusters, resulted in WUE values for yield (WUE_Y), TA (WUE_{TA}) and TSS

(WUE_{TSS}) parameters of approximately 2.6, 2.9, and 3.1 times greater than FI, respectively.

Overall, this study allowed identifying the cumulative adaptation characteristics of the orange trees under study to the application of long-term DI strategies and showing that trees were able to achieve yields and qualitative features similar to those obtained with FI, even after 10 years of application of deficient irrigation regimes.

Keyword: Sustainable citrus production; Semi-arid climate; Orange groves; Water deficit

Introduction

Nowadays, irrigated agriculture has to cope with the increasingly limited availability of water resources, which is becoming worrying especially in arid and semi-arid areas due to climate change (CC) scenarios (Galindo et al., 2018). Indeed, the intensification of drought events expected under the CC scenarios will contribute to the development of stress conditions for crops following the increase in the thermal gradient, the reduction of precipitation, with consequent negative effects on productivity (Fracasso et al., 2020). In the Mediterranean semi-arid environment, the success of citrus production largely depends on adequate irrigation (Rallo, et al., 2017). It is, therefore, essential to manage the available water resources in a sustainable way, in order to optimise the productivity of the citrus groves, while enhancing their adaptation to water shortage conditions.

In this perspective several studies have emphasised the importance of adopting localised irrigation techniques for citrus groves (e.g. surface and sub-surface drip irrigation), in combination with innovative operating methods (e.g. use of very low flow/pressure drippers) and the optimal management of irrigation water (e.g. deficit irrigation, DI, including partial root-zone drying, PRD and regulated deficient irrigation, RDI) (Provenzano et al., 2005; Consoli et al., 2017, Vanella et al., 2018, Vanella and Consoli, 2018). It is known that DI strategies, such as RDI (Gasque et al., 2016; García-Tejero et al., 2010; Consoli et al., 2014) and PRD (Marsal et al., 2008; Romero et al., 2012; Consoli et al., 2017), permit to maintain (or slightly reduce) crop production features by applying a considerably lower amount of irrigation volume, with the consequent increase of crop water use efficiency (WUE) (English, 1990; Consoli et al., 2012; 2014; García-Tejero, et al., 2011; Ballester et al., 2011). The adoption of the RDI technique requires a deep knowledge of the physiological crop response to water deficit conditions, in order to identify the optimal phenological phase for the application of water restrictions in order to minimise the negative effects on crop yield and quality (Navarro et al., 2010; 2015; Aguado et al., 2012, Pérez-Pérez et al., 2009). In particular, several studies have shown that the application of moderate water stress conditions on citrus fruits during the flowering and fruit set phases generally compromises the yield due to the increase in fruit fall (Doorenbos and Kassam, 1979; Ginestar and Castel, 1996; Romero et al., 2006a; Pérez-Pérez, et al., 2008). On the other hand, water restrictions applied during the last phase of fruit growth and ripening decrease yield by reducing fruit weight and size (González-Altozano and Castel, 1999; Treeby et al., 2007a; Pérez-Pérez et al., 2009; García-Tejero et al., 2010), while the period following fruit fall was less sensitive to water restrictions (Cohen and Goell, 1988).

Considering the PRD strategy (i.e., irrigation is alternatively applied to only one half of the root-zone, while the other half is kept dry) (Blackman and Davies, 1985), several studies have shown that in the root system under drying soil, biochemical signals are triggered by the roots to shoot, causing physiological responses for plant adaptation, such as stomatal conductance (g_s) and plant transpiration reduction (Dry et al., 2000; Hutton 2011; Mossad et al., 2018, 2020; Gotur et al., 2018). The main component of the biochemical signalling process involves the abscissic acid hormone (ABA), also known as "plant stress hormone" (Prgomet et al., 2020; Chai et al., 2016). In general, higher ABA concentrations in the roots and in the leaf tissues have been found during the application of severe DI conditions (Romero-Conde et al., 2014; Puglisi et al., 2019) and prolonged PRD, with the consequent increase in the root-shoot ratio and greater WUE (Peréz-Peréz et al., 2018).

Leaf (Ψ_{leaf}) and stem water potential (Ψ_{stem}) are the most significant physiological indicators, capable of monitoring and identifying plant water status (Taylor et al., 2015; Jamshidi et al., 2020). Specifically, Ψ_{stem} measurements are more sensitive to slight differences in plant water status than Ψ_{leaf} as it is less affected by the evaporative demand (and by leaf-to-leaf variations in stomatal opening), and it is more representative of the tree water status than the Ψ of an exposed leaf (Naor, 2006; Ortuño et al., 2006; García-Orellana et al., 2013). Thus, Ψ_{stem} has become a valuable indicator for irrigation scheduling, and has been used as a threshold for both conventional and DI management (Girona et al., 2006a, 2006b). However, Ψ_{stem} refers to a specific moment of the day, requiring also frequent repetitions during the irrigation season for a proper crop monitoring (Ballester et al., 2013; Ortuno et al., 2006). Therefore, alternative water stress indicators are required to overcome these limitations. Sap flow (SF) techniques allow indirectly measurements of plant water losses. In particular, automated SF systems permit to continuously monitor the transpiration activity of both well-watered and deficit irrigated plants (Smith and Allen, 1996; Goldhamer et al., 1999; Ortuno et al., 2004a, 2004b). SF is related to external weather conditions, such as vapour pressure deficit (VPD) (Kang et al., 2003; González-Altozano et al., 2008; Du et al., 2008; Dragoni et al., 2009; Köhler et al., 2010; Motisi et al. 2012), soil water content (SWC) and physiological parameters such as Ψ_{stem} and g_s (Liu et al., 2009; Ortuño et al., 2010).

DI strategies in some cases reduce the fruit quality (Mougheith et al., 1977), mainly influencing the qualitative parameters of citrus production such as total soluble solids and acidity (Chartzoulakis et al., 1999). For example, Romero et al. (2006b) observed a dependency between the decrease in clementine fruit quality and the phenological stage in which DI is applied. Other studies have shown that DI conditions may lead to a greater number of smaller fruits (Treeby et al., 2007b). Conversely, Peng and Rabe (1998) concluded that, in Satsuma mandarin, fruit size was not affected by water restrictions. Therefore, variations in the fruit weight or diameter at any stage of development can depend on the species and change depending on the times and the water restriction imposed by the irrigation regimes.

Although there is a large number of studies conducted in Mediterranean semi-arid climatic conditions, analysing the effects of the adoption of DI strategies on citrus (Ballester et al., 2014; Gasque et al., 2016; Gonzalez-Dugo et al., 2018), is important to explore the physiological, productive and qualitative adaptation characteristics that this type of crop can acquire after a long term DI application. Table 1 reports a number of study cases evaluating the long-term effects of DI on citrus crops.

Table 1. Roundup of studies on deficit irrigation (DI) strategy applied on citrus orchards and main findings

Study	Irrigation treatment	Duration	Crop/Location	Main results
Garcia-Tejero et al., (2011)	Control (C) = 100% ET_c; Sustained deficit irrigation (SDI): SDI-75%; SDI-65%; SDI-50%.	2004-08	Orange trees Guadalquivir river basin, Spain	No significant differences in fruit yield between SDI and C. Significant differences between SDI and C in total soluble solids (TSS) and titratable acidity (TA) and significant relationships between Ψ_{stem} and irrigation. SDI-50% increases the water productivity.
Aguado et al., (2012)	C = 100% ET_c; DI treatment with a water stress ratio of 0.75.	2009-10	'Navelina' orange trees El Campillo, (Huelva), Spain	The final yield was not affected by water deficit. No negative effects were found on organoleptic and nutraceutical fruit properties in both treatments. DI showed higher values of the maturity index (MI) due to the maintenance of the ratio between TSS and TA contents.
Ballester et al., (2014)	C = 100% ET_c; moderate Regulated deficit irrigation RDI-1 applied from mid July to mid September at 50% of C; severe RDI-2 with trees irrigated at 35% of C.	2007-12	Clementina de Nules Valencia, Spain	RDI-2 led to lower fruit size, yield and economic return. RDI-1 did not significantly reduce the cumulative yield and the economic yield compared to C, allowing a water saving of 15%. Overall RDI has greater TSS and TA and reduced the relative trunk growth and weight of the pruning by about 20% compared to C.
Consoli et al., (2014)	C = 100% ET_c; subsurface SSDI = 75%	2011-12	Tarocco Sciara Siracusa, Italy	Moderate to severe water deficit conditions applied in a young orange orchard have

	ET_c; RDI = 50% or 100% ET_c; PRD = 50% ET_c on one side of the root-zone while the other side was kept dry.			permitted water savings up to 41% (PRD) without determining significant negative effects on plants physiology, yield and fruit quality characteristics. Fruit size was reduced in RDI and PRD, which showed higher levels of TSS and TA.
Stagno et al., (2015)	C = 100% ET_c; subsurface SSDI = 75% ET_c; RDI = 50% or 100% ET_c; PRD = 50% ET_c on one side of the root-zone while the other side was kept dry.	2011-12	Tarocco Sciara Siracusa, Italy	SSDI was quite comparable to FI in terms of irrigation performance. PRD and RDI showed difference in terms of measured physiological parameters (g_s and Ψ_{stem}) in comaparison to FI and SSDI, although they were within the sustainable range for citrus orchards
Gasque et al., (2016)	C = 100% of the irrigation dose (ID); RDI 40%/60% ID. During the last 2 years, C at 100%ID and the RDI further reduced by 20% respect to the previous years.	2007-11	'Navelina' orange trees Senyera, Valencia, Spain	Long-term RDI strategies during the summer (initial phases of fruit enlargement) were successfully applied in commercial Navelina citrus trees. No negative effects are observed if a threshold value $\Psi_{stem} = -2.0$ MPa is not exceeded. Mild level of stress lead to water savings around 20% without crop detriment.
Consoli et al., (2017)	C = 100% ET_c; PRD = 50% ET_c on one side of the root-zone while the other side was kept dry alternating the sides every week.	2013-14	Tarcocco Sciara Siracusa, Italy	PRD technique has allowed significant water savings in relation to full irrigation treatment without causing a significant reduction in crop production and improving fruit quality features and WUE.

Gonzalez-Dugo et al., (2018)	C = full water requirements; RDI-1 50%C from mid-June to early September; RD-I2 at 37%C.	2009-12	Orange trees and hybrid mandarin Seville, Spain	Transpiration has been reduced. The tangerine and late orange yields were sustained when irrigation was reduced to 50–55%. The relationship between yield and transpiration indicates that mandarin is less sensitive to water stress than late orange probably due to a change in the dynamics of growth and fruit development.
Mossad et al., (2018)	C at 100%ET$_c$; PRD 50%C only on one alternated side of the root zone; continuous DI at 50%C.	2007-12	Orange trees Palermo, Italy	Tree daily water consumption was significantly lower in PRD and DI than in C. PRD shows more sustainable fruit growth than DI. PRD did not induce any significant advantage in terms of final yield over a simple reduction of irrigation volumes.
Grilo et al., (2019)	C at 100% ET$_c$; PRD 50%ETc to one alternated side of the root-zone; continuous DI= 50% ET$_c$.	2007-13	Orange trees Palermo, Italy	No differences in Ψ_{stem} were found among irrigation treatments. SF density was higher in DI than in C and PRD. In May, fruits of the DI and PRD treatments showed a lower relative daily growth rate (RGR) than fruits of the C treatment due to a possible lack of carbon and nutrients. SF was directly related to fruit RGR at low SF rates, but inversely related to RGR at SF rates.
Mossad et al., (2020)	C at 100%ET$_c$; PRD at 50% ET$_c$ to one alternated side of the root-zone; continuous DI = 50%ET$_c$.	2007-12	Orange trees Palermo, Italy	Yield parameters were generally not affected by PRD, and only yield per tree was lower in DI than C. Fruit size and juice content were reduced by DI, but not by PRD. Both PRD and DI increased juice soluble

				solids and acidity, vitamin C and carotenoid concentrations, as well as sugar productivity per unit of irrigation water.
Silveira et al., (2020)	T100 at 100 %ET_c, T75 at 75 % ET_c, T50 at 50 %ET_c, T25 at 25 % ET_c and T0 rainfed.	2013-18	Grove of Pêra sweet orange Sao Paulo, Brazil	DI improved yield in 3 out of 5 seasons. Yield increments due to irrigation ranged from 15 to 64 %. Full irrigation and DI yielded 30.8% more than rainfed after 5 seasons. DI resulted in lower acidity and higher maturation index compared to rainfed. T50 increases yield, quality and water saving.
Jamshidi et al., (2020)	5 treatments with different irrigation amounts based on the fraction of ET_o : I_{100}, I_{90}, I_{75}, I_{60}, I_{45}.	2016-17	Orange trees Kazeroon, Iran	In the period of high demand for evaporation, the trees of treatments I_{100}, I_{90}, I_{75} reduced their g_s (0.11–0.07 mol m^{-2} s^{-1}) to maintain a relatively constant Ψ_{leaf}, while the trees of treatments I_{60} and I_{45} (severely stressed). Ψ_{leaf} significantly reduced when g_s dropped below 0.07-0.08 mol m^{-2} s^{-1}. I_{60} was recommend for sustainable citrus production.

The main objective of the study was to evaluate the ability of citrus groves (*C. sinensis* (L.) Osbeck), grown in typical semi-arid climatic conditions, to adapt to the application of moderate / severe water deficit regimes after a decade (since its plantation in 2010). In particular, the cumulative adaption characteristics of the orange groves was assessed on a minimum set of physiological (stem water potential - Ψ_{stem} and sap flow - SF measurements) and productive/qualitative indicators considered significant for identifying the water status and water use efficiency features of the crops under study. This purpose was achieved: (i) by comparing the behaviour of DI trees with those under FI strategies during two irrigation seasons (i.e. 2018-19), and (ii) by evaluating whether deficit irrigated trees have changed their response over time.

Materials and methods

Study site and irrigation treatments

The long-term experiment on DI strategies application was carried out in an orange orchard (*Citrus sinensis* (L.) Osbeck, Tarocco Sciara grafted on *Carrizo citrange Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck) located in Eastern Sicily (insular Italy; 37° 20' N, 14° 53' E). The study covered a time phase of the orange groves from 3 to 12 years old, corresponding to the irrigation seasons of the years 2010-19.

The climate of the study site is typical semi-arid Mediterranean, with warm and dry summers, where generally precipitation does not occur (Table 2). Mean air temperature (T_{air}) and mean annual precipitation during the years 2010-19 were about 18 °C and 581 mm, respectively (Ciaccia et al., 2019). A reference weather station, located about 2 km far from the study site and managed by Servizio Informativo Agrometeorologico Siciliano (SIAS), was used for calculating the reference evapotranspiration (ET_0) at the study site (Table 2). Irrigation scheduling was based on the FAO-56 Penman-Montetih (P-M) approach (Allen et al., 1998), adjusted by the crop coefficient (K_c, equal to 0.7 from Consoli et al., 2006a, 2006b), the localised factor (i.e. mean of 0.7, Consoli et al., 2017) and eventually for rain occurrence. SWC measurements were collected at hourly basis at the different irrigation treatments through ECH2O probes (Decagon, Inc., Pullman, WA, USA); data were then averaged at daily scale for monitoring the soil moisture changes and for applying corrections to the calculation of the irrigation dose, when appropriate. Details on the setup of the SWC sensors and soil texture and hydrological characteristics (i.e. sandy loam texture, with field capacity, FC, and wilting point, WP, of 0.28 and 0.14 cm^3 cm^{-3}, respectively) are reported in Consoli et al., (2017), Longo-Minnolo et al. (2020) and Saitta et al. (2020).

Table 2. Irrigation volumes (mm) and water saving respect to full irrigation treatment (%, in brackets) at the irrigation treatments during the long-term deficit irrigation period (2011-2019) (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root drying), seasonal reference evapotranspiration (ET_0), precipitation (P).

Year	FI	SDI	RDI	PRD	ET_0 (mm)	P (mm)
2011	182	150 (17.4%)*	158 (13.2%)*	108 (40.6%)*	700	80
2012	261	210 (19.4%)*	207 (20.6%)*	161 (38.3%)*	900	40
2013	259	154 (40.5%)*	154 (40.5%)*	109 (57.9%)*	703	68
2014	266	240 (10.1%)*	232 (12.7%)*	111 (58.5%)*	730	61

2015	279	204 (26.8%)*	174 (37.6%)*	158 (43.4%)*	697	100
2016	279	213 (24.0%)*	198 (29.0%)*	155 (45.0%)*	723	100
2017	209	158 (24.0%)*	163 (22.0%)*	125 (40.0%)*	783	37
2018	312	248 (20.6%)*	211 (32.4%)*	150 (51.9%)*	596	93
2019	302	250 (17.4%)*	226 (25.4%)*	142 (53.0%)*	636	115

* water saving (%) was defined as [1 – (irrigation in SDI, RDI or PRD/irrigation in FI)] × 100

During the last irrigation seasons 2018-19, irrigation was supplied, following a randomized block design experiment (Figure 1), from mid June (Day of Year, DOY, 169 and 168 in 2018 and 2019, respectively) to the end of September (DOY 273 and 280 for 2018 and 2019, respectively), 3 times per week, early in the morning. The experimental irrigation treatments include: (i) a fully irrigated treatment (FI), where irrigation rate corresponds to 100% of ET_c; (ii) a sustained deficit irrigation treatment (SDI) irrigated at 75% of ET_c; (iii) a RDI treatment (RDI), irrigated at 100% of ET_c, except for the II phenological stage (i.e., fruit growth, starting from DOY_s 204 and 217 in 2018 and 2019, respectively), in which irrigation rate corresponds to 50% of ET_c) and; (iv) a PRD treatment (PRD), irrigated at 50% of ET_c, alternating the use of the dripper lines located at the both sides of the trees trunk with a switching time of 7 days (e.g., Consoli et al. 2014, 2017; Dzikiti et al. 2006; El-Otmani et al. 2020; Sampaio et al. 2010). FI, RDI and PRD were irrigated by a surface drip irrigation system, the SDI with a sub-surface drip irrigation system. Each dripper emitted 4 $L\ h^{-1}$, for a total of 8 $L\ h^{-1}$ in FI, with an operational pressure of 1 bar. DI treatments actually began in 2011, in order to allow in the transplanting year 2010 a regular adaptation of trees in the open field. Table 2 reports the irrigation volumes supplied during the long-term application of the DI strategies at the experimental field (from 2011 to 2019).

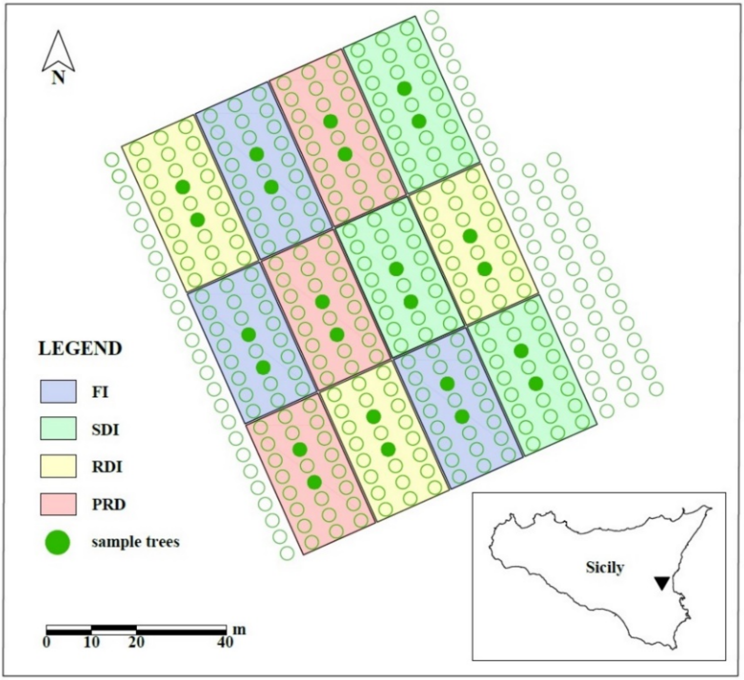

Figure 1. Experimental irrigation design with the location of the sample trees for the monitoring campaigns (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root drying).

The morphological aspects of the trees under study (see sample trees in **Figure 1**), i.e., trunk cross-sectional area (TCSA), canopy diameter and tree heights, showed similar values at the end of the last irrigation season among the irrigation treatments (**Table 3**).

Table 3. Trunk cross-sectional area (TCSA), canopy diameter and plant height and their standard error (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying); different letters indicate statistically significant differences among the irrigation treatments according to Tukey's test ($p \leq 0.05$).

Treatment	**TCSA (cm^2)**	**Canopy diameter (m)**	**Plant height (m)**
FI	183.06 ±19.38 (a)	3.54 ±0.17 (a)	4.48 ±0.34 (a)
SDI	182.06 ±18.04 (a)	3.53 ±0.12 (a)	4.51 ±0.24 (a)
RDI	165.39 ±18.35 (a)	3.35 ±0.11 (a)	4.68 ±0.22 (a)
PRD	166.29 ±17.90 (a)	3.56 ±0.02 (a)	4.48 ±0.05 (a)

Physiological measurements

Ψ_{stem} measurements (MPa) were performed at midday, every 15 days during the irrigation seasons 2018-19, using a pressure chamber (Scholander et al., 1965), on fully exposed mature leaves (2 leaves per tree, 1 tree per treatment and replica), for a total number of 12 leaves per treatment (Figure 1). Leaves were previously wrapped in aluminium foil bags, at least an hour before the measurements.

Semi-hourly SF data obtained using the heat pulse velocity method (Swanson and Whitfield, 1981), were derived using sap flow probes (Tranzflo NZ Ltd., Palmerston North, NZ) installed on 2 trees for each treatment. Details of the adopted SF sensors and the descriptions of the methodological approaches are reported in Consoli et al. (2014, 2017) and Saitta et al. (2020). SF data were aggregated at hourly and daily scales. For daily scale, only daylight values were considered.

Crop yield and qualitative characteristics

Yield and qualitative fruit characteristics were analysed during the 2019-20 harvest periods. Fruits were collected by sampling each tree for treatment and replica (for a total number of 24 trees) to determine the number of fruits and their average weight. The equatorial section (ED, mm) was measured through a calibre tape.

For sampling, 10 fruits per tree were selected (2 trees per treatment and replica, for a total number of 24 trees and 60 samples per treatment, Figure 1) for determining the fruit main qualitative parameters (i.e. total soluble solids, TSS; and titratable acidity, TA). TSS was obtained with a digital temperature compensated refractometer (Atago RX5000 Co. LTD Washington, US State), while TA was determined on the orange juice by titration with 0.1 N NaOH. The maturity index (MI) was expressed as the ratio between TSS and TA.

Water use efficiency calculation

In order to evaluate and compare the impact of the different DI regimes on the productive features of the orange trees, the water use efficiency (WUE_Y) was determined as the ratio between yield (kg) and irrigation volume applied per unit area (mm ha^{-1}) (Heydari, 2014; Fernández et al., 2020). Similarly, WUE was estimated on the basis of the qualitative features as the ratio between TA and TSS (g) and irrigation volume distributed (m^3), named as WUE_{TA} and WUE_{TSS}, respectively.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out on physiological and qualitative/productive dataset for assessing the differences among the irrigation treatments. A post-hoc analysis based on Tukey HSD test (Tukey Honest Significant

Differences) was performed at significance level (p value) of 0.05, 0.01 and 0.001, respectively. In addition, the strength and direction of VPD/SF and T_{air}/SF relationships were identified by Spearman rank order (rho) and Pearson (r) correlation analyses. The above-mentioned statistical analyses were performed using Minitab software package (v. 16, Minitab, Inc., State College, PA, USA).

Results

Long-term physiological responses of orange orchards to water deficit

Figure 2 shows the average Ψ_{stem} values (MPa) measured at the irrigation treatments during 2018 and 2019 irrigation seasons. FI and RDI showed similar average Ψ_{stem} values, ranging from -1.36 to -1.62 MPa, with the exception of DOY 225 (2018) when RDI presented significantly lower Ψ_{stem} values than FI (-1.46 and -1.57 for FI and RDI, respectively). The differences between the two treatments in terms of Ψ_{stem} are evident and when the RDI was supplied with 50% of ET_c. RDI treatment was irrigated at full (i.e. 100% of ET_c) until DOY 204 and DOY 217 in 2018 and 2019, respectively. SDI and PRD evidenced more negative Ψ_{stem} values than FI, with values varying between -1.37 and -1.78 MPa in 2018 and between -1.56 and -2.04 in 2019.

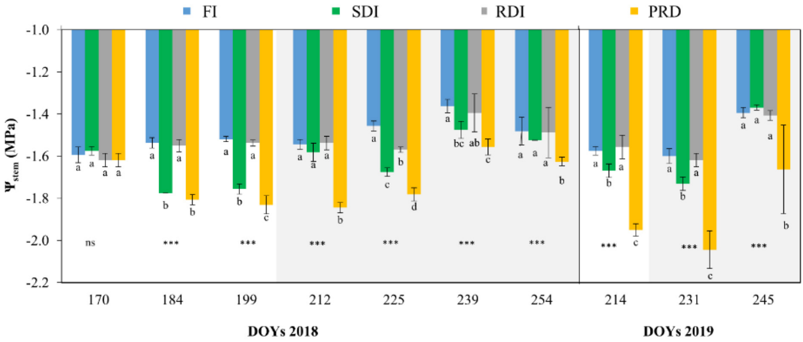

Figure 2. Average Ψ_{stem} values (MPa) measured at the irrigation treatments during 2018 and 2019 irrigations seasons (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). Shaded regions indicate when RDI was applied. The error bars indicate the standard deviation of the average. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$). Significant p values ($p \leq 0.05$, 0.01 and 0.001) are indicated with *, ** and ***, respectively; ns indicates not significant differences.

Figure 3 shows the different clusters obtained by plotting Ψ_{stem} values and their corresponding hourly SF data. Note that the clusters present specific distributions, mainly as function of the different SF rates due the different degree of water deficits applied. In particular, the SF - Ψ_{stem} cluster for FI is mainly located in the upper right part of Figure 3, denoting higher SF rates (from 0.141 to 0.165 mm h^{-1}) with low water stress effects (Ψ_{stem} ranging from -1.3 to -1.6 MPa). For SDI and RDI the SF - Ψ_{stem} clusters were distributed between similar Ψ_{stem} values from -1.3 to -1.8 MPa, and in PRD the SF - Ψ_{stem} clusters were recognized between Ψ_{stem}

ranging from -1.6 to -1.8 MPa (with few exceptions which presented Ψ_{stem} values lower than -1.9 MPa). However, these DI treatments presented different SF fluxes, with RDI having the lowest values (0.076-0.087 mm h^{-1}), followed by SDI (0.100-0.106 mm h^{-1}) and PRD (0.115-0.131 mm h^{-1}), respectively.

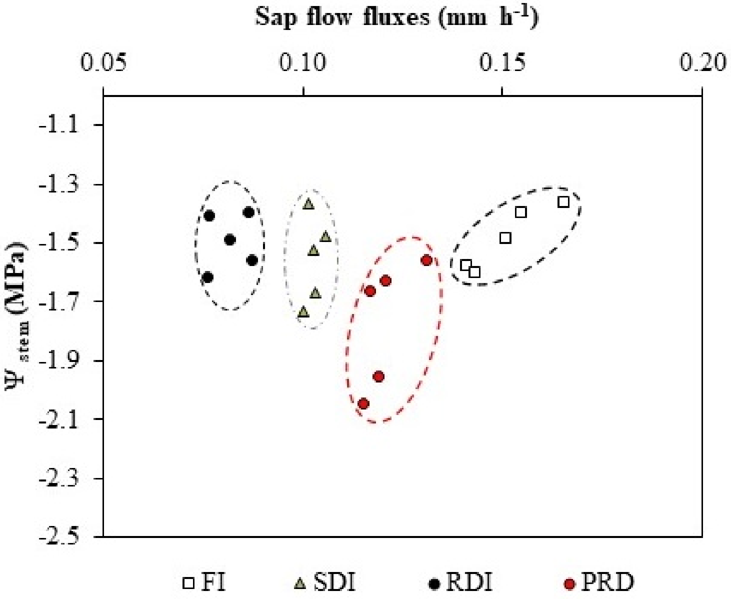

Figure 3. Sap flow (SF) – Stem water potential (Ψ_{stem}) clusters at the different irrigation treatments (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying).

Physiological adaptation to abiotic conditions

Figures 4 shows the relationship between VPD and SF observed during 2018-2019 at hourly (daylight) scale. Two different stages can be differentiated in the SF-VPD relationship. The first one corresponded with a linear increasing pattern, for VPD values ranged between 0 and 2 kPa. At this stage, FI presented a significant higher slope term (0.058), followed by PRD (0.042) and SDI (0.037), respectively, being RDI the treatment with the lowest slope term (0.035) (Figure 4).

Once this VPD threshold was exceeded (i.e. 2 kPa), the relationship between VPD and SF tended to become more stable, until reaching a certain point (SF_{max}) where VPD hardly influenced SF. SF_{max} depended also on the irrigation treatment. Thus, whereas SF_{max} was found approximately at 0.14 mm h^{-1} for FI, DI treatments obtained lower values, being 0.11, 0.09 and 0.07 mm h^{-1} for PRD, SDI and RDI, respectively.

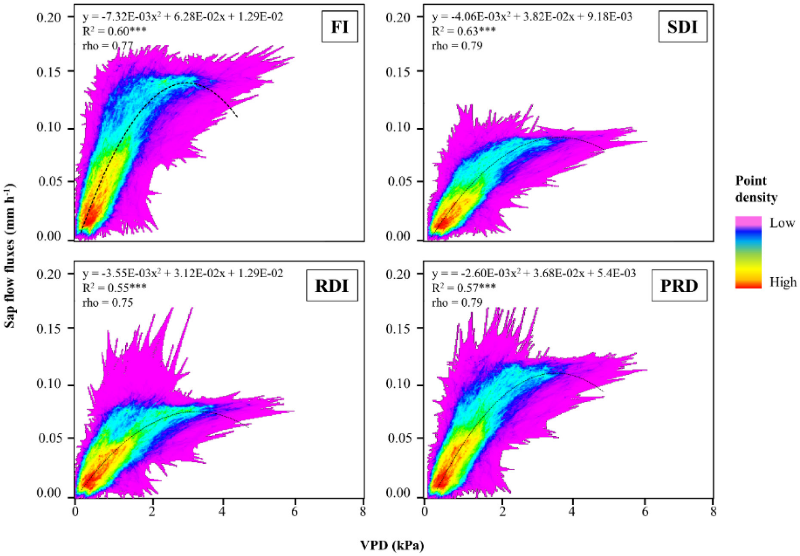

Figure 4. Hourly correlation between vapour pressure deficit (VPD) and sap flow fluxes (SF) for the period 2018-2019 at

the different irrigation treatments (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). The significance of Spearman rank order (rho) correlation values is also reported (ns: not significant; *, **, ***: significant at $p \leq 0.05$, 0.01 and 0.001, respectively).

The relationship obtained during 2018-2019 between the hourly SF and T_{air} values (Figure 5) followed almost linear patterns, with R^2 ranging from 0.58 (RDI) to 0.74 (SDI). As for the hourly SF and VPD relationship, the SF and T_{air} trends showed different magnitudes (i.e. slope terms) in relation to the adopted irrigation strategy. In this sense, FI presented the higher slope term (= 5.33E-03), followed by PRD (= 4.06E-03) and SDI (= 3.46E-03), respectively, being RDI the treatment with the lowest slope term (= 2.58E-03).

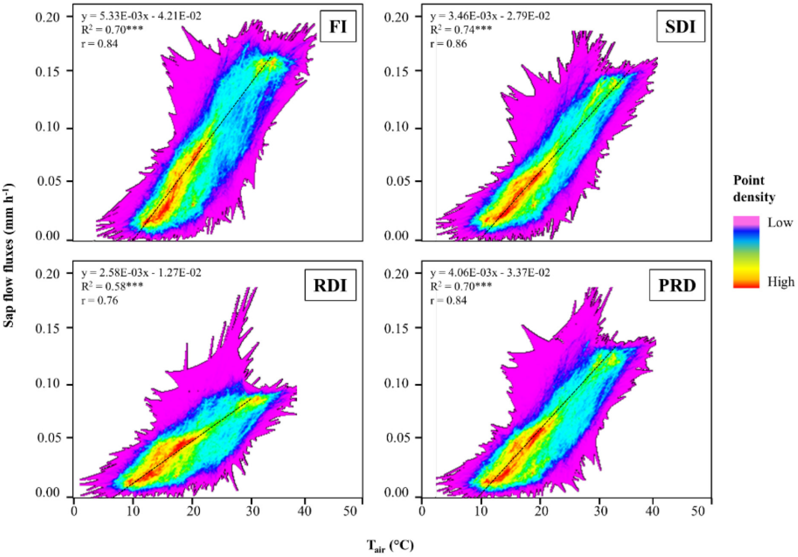

Figure 5. Hourly correlation between air temperature (T_{air}) and sap flow fluxes (SF) for the period 2018-2019 at the different irrigation treatments (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). The significance of Pearson (r) correlation values is also reported (ns: not significant; *, **, ***: significant at $p \leq 0.05$, 0.01 and 0.001, respectively).

Similar correlation trends to those obtained at hourly scale for VPD and T_{air} with SF were observed also at daily scale, but with higher R^2 values (0.68-0.74 vs 0.55-0.63 for SF-VPD at daily and hourly scale, respectively; and 0.75-0.83 vs 0.58-0.74 for SF-T_{air} at daily and hourly scale, respectively) (Figure 6). Specifically, for daily mean SF-VPD relationships (Figure 6a), it can be observed that SF_{max} values were 1.4, 1.1, 0.9 and 0.8 for FI, PRD, SDI and RDI, respectively. Regarding the daily mean SF-T_{air} relationships (Figure 6b), the slope terms followed the same relative pattern as at hourly scale (Figure 5), ranging from 0.05 (for FI) to 0.02 (for RDI).

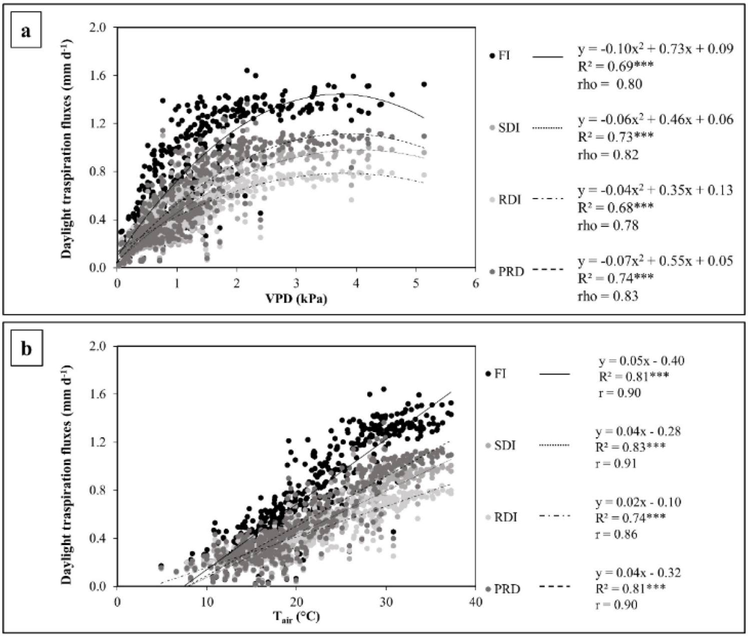

Figure 6. Daily mean correlation between (a) vapour pressure deficit (VPD) and (b) air temperature (T_{air}) with sap flow fluxes (SF) for the period 2018-2019 at the different irrigation treatments (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). The significance of Spearman rank order (rho) and Pearson (r) correlation values are also reported (ns: not significant; *, **, ***: significant at $p \leq$ 0.05, 0.01 and 0.001, respectively).

Figure 7 shows the changes in SWC observed in the upper soil layer (0.10-0.30 m) during the 2018 and 2019 irrigation seasons. As expected, all treatments had daily SWC values ranged between the FC and the WP. In particular, all treatments were characterised by similar average daily SWC conditions, ranging between 0.22 (for SDI and PRD) to 0.24 cm^3 cm^{-3} (for FI and RDI) (Table 4). When comparing the SWC trends of the different irrigation treatments it was evidenced that they differed in the amplitude of the drying and wetting cycles, which were more pronounced in SDI and especially in PRD treatment. Despite these differences, a constant SWC trend along the irrigation seasons were observed for all treatments.

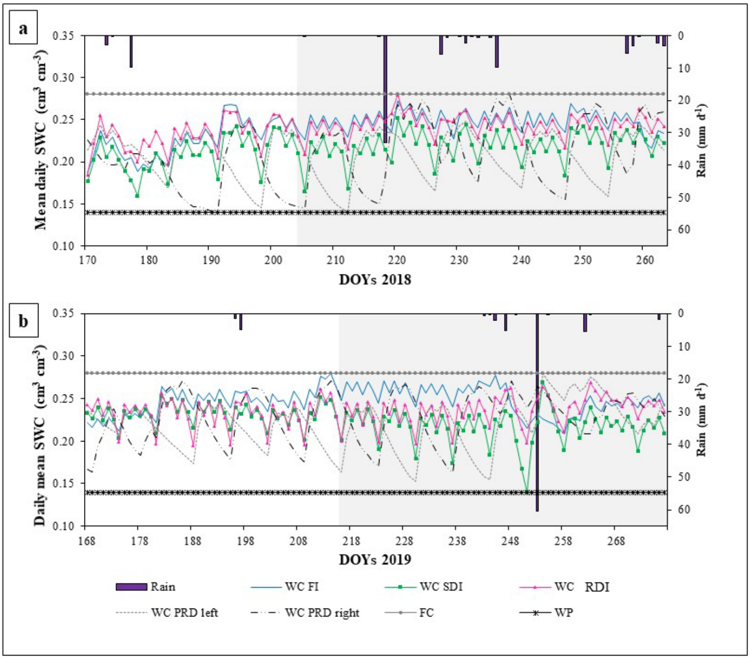

Figure 7. Daily mean soil water content (SWC, cm^3 cm^{-3}) for (a) 2018 and (b) 2019 irrigation seasons at the different irrigation treatments (FI = full irrigation; SDI = sustained

deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root–zone drying).

Table 4. Daily mean values of soil water content (SWC, (cm^3 cm^{-3}) (with standard deviation in brackets), cumulative irrigation and rain (mm) per treatment during irrigation seasons 2018-19. (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying).

		FI	**SDI**	**RDI**	**PRD**	**Rain (mm)**
2018	**SWC** (cm^3 cm^{-3})	0.24 (0.02)	0.22 (0.02)	0.24 (0.02)	0.21 (0.02)	78
	Irrigation (mm)	311	248	211	150	
2019	**SWC** (cm^3 cm^{-3})	0.25 (0.02)	0.22 (0.02)	0.24 (0.02)	0.22 (0.01)	83
	Irrigation (mm)	301	250	224	141	

Crop yield, qualitative and WUE features

In 2019, total yield varied from 25.5 to 31.7 t ha^{-1}, and no significant differences were observed among the different irrigation treatments (Table 5). Fruit weight, number of fruits and ED, also without differences among the irrigation treatments, ranged from 249 to 280 g, from 286 to 434 and from 77 to 79 mm, respectively (Table 5). The yield parameters obtained in 2020 were similar to those obtained in 2019 (Table 5). Specifically, total yield ranged from 29.9 to 34.5 t ha^{-1}, fruit weight from 243 to 289 g, number of fruit from 302 to 407 and ED from 78 to 82 mm.

Table 5. Effect of irrigation treatments on yield parameters (yield, fruit number and weight and equatorial section, ED) and their standard error for 2019 and 2020 (2018 and 2019 irrigation seasons) (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). Different letters indicate statistically significant differences among the irrigation treatments for each year according to Tukey's test ($p \leq 0.05$).

Year	**Treatment**	**Yield (t ha^{-1})**	**Fruit weight (g)**	**N. of fruits**	**ED (mm)**
2019	FI	25.5 ± 1.3 (a)	280 ± 9 (a)	286 ± 22 (a)	79 ± 1 (a)
	SDI	31.7 ± 1.6 (a)	252 ± 12 (a)	434 ± 27 (a)	77 ± 1 (a)
	RDI	27.9 ± 2.2 (a)	268 ± 10 (a)	358 ± 37 (a)	79 ± 1 (a)
	PRD	26.6 ± 1.7 (a)	249 ± 8 (a)	398 ± 31 (a)	77 ± 1 (a)
2020	FI	30.1 ± 4.7 (a)	289 ± 28 (a)	302 ± 33 (a)	82 ± 2 (a)
	SDI	34.5 ± 2.4 (a)	277 ± 15 (a)	407 ± 23 (a)	82 ± 2 (a)
	RDI	31.3 ± 1.4 (a)	243 ± 14 (a)	336 ± 33 (a)	78 ± 2 (a)

PRD	29.9 ± 2.1 (a)	245 ± 12 (a)	353 ± 31 (a)	78 ± 2 (a)

The quality parameters, i.e., TA (g L^{-1}), TSS (°Brix) and MI, referring to the 2019 and 2020 harvests (2018 and 2019 irrigation seasons) are reported in Table 6. Referring to TA, similar values were obtained for both years, varying between 1.1 and 1.3 g L^{-1}. The same behaviour was observed for TSS and MI, with values ranging from 10.1 to 11.2 °Brix and from 8.6 to 9.8 for both harvest periods, respectively. Additionally, despite the different levels of water deficit applied, no significant differences were observed in qualitative terms between the different irrigation treatments.

Table 6. Effect of irrigation treatments on fruit quality parameters (titratable acidity, TA; total soluble solids, TSS; and maturity index, MI) and their standard error for the 2019 and 2020 harvest (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). Different letters indicate statistically significant differences among the irrigation treatments for each year according to Tukey's test ($p \leq 0.05$).

Year	Treatment	TA (g L^{-1})	TSS (°Brix)	MI
2019	FI	1.2 ± 0.1 (a)	10.8 ± 0.2 (a)	9.1 ± 0.4 (a)
	SDI	1.2 ± 0.0 (a)	11.1 ± 0.1 (a)	9.0 ± 0.3 (a)
	RDI	1.2 ± 0.1 (a)	11.2 ± 0.2 (a)	9.3 ± 0.5 (a)
	PRD	1.3 ± 0.1 (a)	11.1 ± 0.2 (a)	8.8 ± 0.3 (a)
2020	FI	1.2 ± 0.0 (a)	10.6 ± 0.2 (a)	9.1 ± 0.3 (a)
	SDI	1.2 ± 0.1 (a)	10.1 ± 0.6 (a)	9.0 ± 0.6 (a)
	RDI	1.2 ± 0.1 (a)	10.4 ± 0.2 (a)	8.6 ± 0.3 (a)

PRD	1.1 ± 0.0 (a)	10.9 ± 0.2 (a)	9.8 ± 0.4 (a)

Table 7 reports the WUE obtained for the productive (yield, WUE$_{Y}$) and qualitative features (TA, WUE$_{TA}$, and TSS, WUE$_{TSS}$) at the different irrigation treatments during the 2019 and 2020 harvest periods (2018 and 2019 irrigation seasons). In particular, in 2019 the WUE$_{Y}$ ranged from 8.2 to 17.7 kg m^{-3}, whereas values obtained in 2020 were slightly higher, varying from 10.0 to 21.2 kg m^{-3}. When comparing the WUE$_{Y}$ values obtained for the different irrigation treatments, significant differences were obtained between FI (8.2 and 10.0 kg m^{-3} for 2019 and 2020, respectively) and PRD (17.7 and 21.2 kg m^{-3} for 2019 and 2020, respectively). Regarding WUE$_{TA}$, the obtained values ranged from 47.7 to 120.7 g m^{-3} for 2019; and from 66.6 to 138.8 g m^{-3} in 2020, being WUE$_{TA}$ for PRD significantly higher than those referring to the other irrigation treatments for the harvest periods. Finally, WUE$_{TSS}$ ranged from 433 to 1,047 g m^{-3} in 2019, whereas higher values were obtained in 2020, varying from 601 to 1,346 g m^{-3}. As for WUE$_{TA}$, significant differences were found for WUE$_{TSS}$ between PRD and the other irrigation treatments.

Table 7. Effect of irrigation treatments on water use efficiency on yield (WUE$_{Y}$) and titratable acidity (WUE$_{TA}$) and total soluble solids (WUE$_{TSS}$) and their standard error in 2019 and 2020 (FI = full irrigation; SDI = sustained deficit irrigation; RDI = regulated deficit irrigation; and PRD = partial root-zone drying). Different letters indicate statistically significant differences among the irrigation treatments for each year according to Tukey's test ($p \leq 0.05$).

Year	Treatment	WUE$_Y$ (kg m^{-3})	WUE$_{TA}$ (g m^{-3})	WUE$_{TSS}$ (g m^{-3})
2019	FI	8.2 ± 0.8 (b)	47.7 ± 4.7 (b)	433 ± 42 (c)
	SDI	12.8 ± 0.4 (ab)	81.2 ± 10.0 (b)	726 ± 77 (b)
	RDI	13.2 ± 2.0 (ab)	81.3 ± 14.1 (b)	745 ± 123 (b)
	PRD	17.7 ± 1.9 (a)	120.7 ± 15.3 (a)	1047 ± 113 (a)
2020	FI	10.0 ± 1.3 (b)	66.6 ± 9.7 (b)	601 ± 85 (b)
	SDI	13.8 ± 2.2 (ab)	83.8 ± 13.1 (b)	726 ± 96 (b)
	RDI	14.0 ± 0.6 (ab)	95.2 ± 5.3 (b)	817 ± 39 (b)
	PRD	21.2 ± 1.8 (a)	138.8 ± 10.2 (a)	1346 ± 92 (a)

Discussion

In this study, the adaptation effects related to the adoption of DI strategies applied over a decade in an orange orchard have been explored through the analysis of a dataset composed of physiological information (i.e., including the main representative plant water status indicators) and yield/qualitative parameters with reference to two last irrigation seasons (2018-2019), which are influenced by a decade of application of DI strategies. The different amount of irrigation volumes supplied during the long-term DI application at the study site (Table 2) resulted mainly dependent on the year-specific climatic conditions and on the progressive growth of the orange trees (i.e., affecting the localised factor and the K_c values).

Previous studies conducted at the experimental site under study have intensively analysed the specific effects due to the application of different levels of water deficit conditions on different physiological parameters, such as, g_s, Ψ_{stem}, SF, leaf photosynthesis, and canopy temperature (i.e. for the irrigation seasons 2011-12 in Consoli et al., 2014; for 2011-12 in Stagno et al., 2015; for 2013-14 in Consoli et al., 2017; for 2015 in Puglisi et al., 2019; for 2018 in Pappalardo et al., 2019). From these studies, Ψ_{stem} and SF were identified as physiological proxies due to their ability to detect the responses to water deficit among the irrigation treatments under study. As shown in Puglisi et al., (2019) for orange orchards growing in Mediterranean semi-arid conditions, the mean Ψ_{stem} values for FI (full irrigation) showed a similar trend to RDI, presenting almost constant values during the irrigation season, both before and after the application of the water restriction (Figure 2). This decrease in Ψ_{stem} differences over time, between the DI treatments (specifically RDI) and FI, could be directly ascribed to the adaptation features developed by the crop, following the long term DI application, since the influence of phenology and the crop size had a minimal effect on Ψ_{stem} behaviour. In fact, Ψ_{stem} measurements were carried out always during the same phenological stage (from mid-June to September) and, the main morphological parameters were quite constant during the last irrigation seasons (Table 3) and stable during the years because trees were commonly subjected to a minimal harvest. A simple evaluation of the Ψ_{stem} values observed after intense rain events has permitted to determine the Ψ_{stem} behaviour under “no water stress” conditions at the study site. In particular, the Ψ_{stem} value in FI after a rain event of 33 mm (DOY 217, 2018, Figure 7) was around -1.46 MPa. Focusing also during other rain events, occurred during previous irrigation seasons, similar Ψ_{stem} values were reached, as for instance in Consoli et al., (2014), with Ψ_{stem} values of -1.4 - -

1.5 MPa in FI after rain events of 8 and 13 mm, on DOYs 224 and 247, 2012, respectively. Similarly, Consoli et al. (2017) found Ψ_{stem} values of about -1.6 MPa after a rain event of 37 mm on DOY 260, 2013. Finally, after a rain event of 43 mm on DOY 223-224, 2015 (Puglisi et al., 2019), Ψ_{stem} values of -1.3 MPa were measured. These findings support the hypothesis that FI properly corresponded with a close-to-non water stress conditions, since Ψ_{stem} values measured in FI during the study period were within this range of values. The Ψ_{stem} behaviour at FI condition is reflected in SWC (Figure 7), being this treatment characterized by higher SWC values than the other DI treatments, thus showing less resistance to root water uptake. This was confirmed also by the SF observations (Figures 3, 4, and5). In comparison to FI, SDI showed slightly more negative Ψ_{stem} values according to the lower SWC condition (Figure 7). Other studies have shown a simultaneous reduction in Ψ_{stem} and g_s rates due to the SWC deficit (Ballester et al., 2013; Garcia-Tejero et al., 2011; Gasque et al 2016). The lower SWC condition observed at SDI in comparison to FI and RDI is probably due to a higher root density concentrated in the most upper part of the soil, until 40 cm of depth, where the SDI drip lines are located. This hypothesis was supported by visually observations carried out on excavated pits that showed higher concentration of fine roots covering the buried drips in SDI than in FI and RDI soil profiles. As expected, the lowest Ψ_{stem} values (Figure 2) were recorded in the most severe DI treatment (PRD) (DOY 214 and 231 in 2019, with values of -1.95 MPa and -2.04 MPa respectively, Figures 2-3), in which the SWC reached the lowest values (Figure 7). However, the general trend observed for SWC, always ranged between the

WP and FC, during the irrigation seasons at all treatments (Figure 7), demonstrating the suitability of the adoption of the FAO-56 ET_c method for scheduling the irrigation in combination with the use of site-specific adjusting parameters for taking into account the crop and soil characteristics. This was also confirmed by no drainage patterns observed in the soil profile by electrical resistivity imaging technique (Vanella et al. 2020).

Overall, as shown in the SF - Ψ_{stem} clusters (Figure 3), crops were able to maintain a certain degree of water stress. Our results proof that over time the citrus grove has been adapted to water stress, without affecting yield and quality characteristics (Tables 5-6). Similar Ψ_{stem} values have been observed by Ballester et al. (2014) and Gasque et al. (2016), who showed that Ψ_{stem} values in citrus should not exceed -1.5 MPa and -2.0 MPa, respectively, in the Mediterranean semi-arid environment.

As for the Ψ_{stem}, the transpirative response was able to reflect the DI conditions occurring at the study site as function of the different magnitude of the applied water deficit and the evaporative demand, (see, the correlations between the SF and the climatic parameters, VPD and T_{air}, Figures 4 and 5), which coincides with the findings of Saitta et al. (2020). These results are in accordance with several researches, showing a general decrease of the SF rates due to the application of DI strategies (Espadafor et al., 2017; Lopez-Lopez et al., 2018; Roccuzzo et al., 2014a, b). Herein, it is possible to note that the SF rates of the DI treatments were lower than the SF fluxes observed in FI, which depends on the level of accumulated water stress throughout the day and environmental demand. A peculiar trend was observed in

PRD, in which 50% of the irrigation volume of FI was applied, that showed SF fluxes greater than the other DI treatments (SDI and RDI). This is probably due to the PRD operational mode that used alternating two dripper lines located at the eastern and western side of the trees. This permits the root system to explore a larger soil volume and to have access to more water resources (Mingo et al., 2004), that otherwise they would not be able to reach. In this sense, Mary et al. (2019) observed privileged direction of root development in PRD, with greater rooting extent in comparison to full rate conditions. In addition, according to Chai et al. (2016), the application of the PRD strategy causes a compensatory effect on SF fluxes. Specifically, the SF rate tends to increase when the roots have directly access to water (wetted area), whereas SF is reduced when the root system is in dry conditions (dry area) with poor access to water. The alternation of the wet/dry portions due to the PRD strategy contributes to maintain, as in our case, higher SF rates. In accordance to other studies (e.g. Kang et al., 2003; González-Altozano et al., 2008; Du et al., 2008; Dragoni et al., 2009; Köhler et al., 2010), a close relationship between the SF fluxes and the weather factors was observed (such as VPD and T_{air}). However, a great variability of the SF rates for any VPD was observed, mainly due to the stomatal adjustment, as shown in Figures 4 and 5. In particular, it can be noted that FI is characterised by higher SF-VPD and SF-T_{air} positive trends than the DI treatments (SDI, RDI and PRD). This behaviour is mainly due to the applied DI conditions, that for T_{air} and VPD increasing, may lead to stomatal closure and therefore to SF rates reduction (Consoli et al., 2014). Stomatal closure in response to the VPD increases could be an effective strategy to avoid excessive water loss in drought conditions and prevent that the Ψ_{stem} falls to dangerous water stress levels (Tyree and Sperry, 1988; Granier et al., 2000) (Figure 3).

From the crop productivity point of view, previous studies carried at the same experimental orchard have shown statistically significant differences among the different DI treatments and FI (Consoli et al., 2014; Consoli et al., 2017), mainly showing slighter fruit weight for the DI trees. For the last two irrigation seasons under study, the productive features (i.e., yield parameters, fruit weight, number of fruits and ED) did not show significant differences among the irrigation treatments, as also reported in Puglisi et al., (2019). This could indicate that plants have adapted over time to these stresses, eliminating the differences in terms of production compared to the FI. These evidences are also confirmed by the similar morphological characteristics obtained among the treatments under study (Table 3). Similar results were obtained by Garcia-Tejero et al. (2011) and Aguado et al. (2012), who showed that the DI application conditions did not influence the final yield for citrus in comparison to the well irrigated conditions. However, Adu et al. (2018) argued that yield response under water-saving irrigation strategies is context-specific and mainly depends on crop species and soil texture.

Water productivity has become a key requirement in sustainable crop production and environmental management (Adu et al., 2019). The applied water deficit in fruit crops has been associated with WUE increasing, not only in terms of WUE_Y (García-Tejero et al., 2010; Carr, 2012), but also considering WUE_{TSS} and WUE_{TA}. In general, the effects of

DI strategies on the fruits qualitative characteristics have been studied in a wide range of crop types, including olives (Wahbi et al., 2005), grapes (Salon et al., 2004) and various citrus varieties, such as Valencian orange (Castel and Buj, 1990), lemon (Sánchez Blanco et al., 1989), mandarin (Peng and Rabe, 1998). Within the qualitative aspects, TSS and TA contents increases represent a consolidated response to water deficit in citrus fruits, even if the water deficit is applied during the whole season or during fruit growth stages (Hutton et al., 2007, 2011; Garcia-Tejero et al., 2010). A meta-analysis on the effects of FI and DI on quality attributes conducted by Adu et al. (2019) reported that DI strategies reached higher improvements than FI when considering improvement in TSS without significantly altering TA or pH of fruits. The increase of TSS and TA in response to water deficit is a very well established response in citrus fruits, particularly when the water deficit is imposed during the whole season (Hutton et al., 2011) or during some fruit growth phases (Hutton et al., 2007; Garcia-Tejero et al., 2010). For example, in studies conducted by Hutton et al. (2011) on navel orange, the concentrations of TSS and acid (g of citric acid / 100 ml of juice) were higher in trees treated with PRD with values respectively of 13.9 and 1.59 compared to normally irrigated trees whose values were respectively 13.5 and 1.42.

Conversely to previous studies conducted in the same orchard where the TSS values for SDI, RDI and PRD were respectively 1.02, 1.11 and 1.10 times higher than the FI treatment (Consoli et al., 2014; Consoli et al. , 2017) and according to studies conducted by other authors (Ballester et al., 2014; García-Tejero et al., 2010; Yakushiji et al., 1996;

Mossad et al., 2020), which found a significant increase of TSS and TA in the context of DI strategies, in this study no statistically significant differences were observed between DI and FI treatments in terms of quality parameters.

This evidences the adaptation effects of the citrus orchard under study due to the application of the long-term DI strategies. The mitigation of the differences in crop quality and production features among the DI treatments and the control over the years can be interpreted as an adaptation measure adopted by the plants for facing the water deficit conditions for increasing stress tolerance, including molecular mechanisms and concomitant growth adjustment (Osakabe et al. 2014). These latter include stomatal responses, ion transport, activation of stress signalling pathways (i.e. ABA, Puglisi et al. 2019), and response to reduce photosynthetic activity. This adaptation effect is also interpreted in WUE terms, (Table 7), which shows higher values for DI treatments (SDI, RDI and PRD) and increases over time with higher WUE values for 2019 compared to 2018. This increase over time is evident by comparing the WUE values of each irrigation treatment for the study years 2018-2019 with the lower values reported in Consoli et al. (2014, 2017) for the same experimental site. In particular, PRD, in which the most severe water restriction was imposed, showed the highest WUE values highlighting the advantages of the PRD adoption for obtaining significant water savings (up to 50%). This result is in accordance to most of the studies carried out on PRD strategies in the context of fruit trees that have experienced higher WUE values in DI conditions, being the applied irrigation volumes the critical component for the WUE increasing (Santos et al., 2005; Spreer et al., 2009).

In addition, the application of the PRD in areas with reduced water availability is useful not only to guarantee water savings, improve the use of nutrients and increase/maintain yield, but also for the production of foods with better characteristics nutritional and health (Jovanovic et al., 2018). Finally, the results of this study contribute to describe the effects on crop characteristics due to the long-term DI conditions, highlighting the success of the applications of the DI strategies, in terms of yield, quality and water savings for citrus crops in Mediterranean semi-arid environments where water resources are limited such as in Sicily.

Conclusion

The main conclusion that can be drawn from this study are reported, as follows:

- the long-term DI strategies can be successfully applied to high-value cropping systems, like orange orchards, in semi-arid climatic conditions;
- overall, from the observed SF - Ψ_{stem} clusters, crops were able to maintain a certain degree of water stress without causing negative effects on the yield and fruit quality;
- the DI treatments have modified the relationships that link the SF-VDP and SF-T_{air}, showing lower trends compared to the fully irrigated treatment;
- the application of the DI strategies allowed water savings up to 53% for the PRD treatment, with consequent increases in the WUE, without compromising yield and fruit main quality parameters (TA and TSS).

The present study suggests the usefulness of starting the application of DI to young citrus plants, from the beginning of their transplantation in the open field, in order to favour the progressive adaptation of the crop to the imposed water regimes. This long-term study sets the basis for the transfer of research knowledge about DI application to the
real citrus context.

9. Main conclusions of the PhD Thesis

This PhD work has allowed us to analyze the physiological, biochemical and production responses, for one of the most important plant species in the Mediterranean region, Citrus sinensis L. (cv Tarocco Sciara grafted on Citrange Carrizo), in the context of deficit irrigation strategies (case study 1-2), also providing interesting suggestions about the long-term applications of these strategies to the real context of citrus fruits (case study 4).

Furthermore, the research has allowed to evidence new information regarding the performance of the different methods for the determination of ETc (case study 3).

For the case study 1, the results show that the application of DI strategies does not cause significant physiological differences in the plants monitored under the different irrigation treatments. A very interesting aspect concerns the contents of leaf ABA which have remained almost constant in all irrigation treatments. This is because in our open field conditions several abiotic stress situations are very likely to occur simultaneously, so factors such as water stress would result in a foliar accumulation of ABA, and factors such as heat stress would inhibit it (Zandalinas et al., 2016). The accumulation of proline in the leaves was not related to the water deficit since its accumulation was found in well irrigated treatments (SDI and SSDI), and as demonstrated in Mattioli et al., (2009) the accumulation of this amino acid could occur even in non-stressed physiological conditions for developmental purposes.

As regards the technological aspect of the irrigation system, SSDI was quite comparable, in terms of performance, with SDI; the behavior of trees growing under SDI (full irrigation) and SSDI (which provides 75% of ETc) was quite similar with the advantage of the latter to also guarantee water savings of approximately 27%.

In case study 2, among the monitored parameters, the photosynthetic rate of the plant seems to be among the physiological parameters less subjected to water stress and high temperatures; a factor that would allow the crop to cope with water restriction during the II phase of growth of the fruit that requires a good water condition of the plant.

In case study 3, the effects of water deficit overall influenced both the performance of the different approaches (FAO-56 and EC) by reducing the ETc rates at the field scale and the transpiration flows at tree level where they were always lower in DI compared to the correspondents measured in FI.

The results obtained from the FAO -56 approach showed that both the single and dual Kc provided similar estimates of ETc, whose values however tended to overestimate the ETc compared to ET_{EC}. Important aspects emerge from the work regarding the use of one approach rather than another.

Although the FAO-56 model is considered among the simplest and cheapest approaches, the estimates derived from this method must be considered with caution as they necessarily imply some generalizations that may not reflect the specificities of the site.

With regard to the choice of an EC system, however, attention must be paid to some precautions before proceeding with its installation on site. In addition to taking into account the greater economic investment necessary for the installation of the detection sensors, it is essential to evaluate the conditions of the experimental site. Generally we recommend the installation of the EC station for large companies, or for a group of small consortium companies falling into a homogeneous area.

The study, also, clarifies the impossibility of indicating in an absolute sense the most appropriate method for determining ET_C, but the selection will depend on the specific characteristics of the crop and the study site.

Case study 4 made it possible to identify the adaptability of orange trees to the application of long-term DI strategies on the basis of a minimum, but absolutely significant, set of physiological indicators (Ψ_{stem} and SF measures) and on production / qualitative parameters (eg weight, number of fruits, TA and TSS content). Differently from the results obtained in previous studies conducted on the same experimental site, in this study it was possible to observe negligible differences between the treatment of full irrigation (FI) and the treatments of deficit irrigation in cumulative terms after a decade of application of the same, indicating that the crop has adapted well to the imposed water regime.

Therefore it is advisable to apply these DI strategies to orange plants from the beginning of their transplantation in the open field, in order to favor a gradual and progressive adaptation of the crop to the irrigation strategies applied.

Overall, the results obtained in this PhD work allow us to affirm that DI strategies are valuable tools in order to improve the WUE, obtaining significant water savings and maintain sustainable production levels especially in those areas where agriculture is heavily dependent on irrigation.

References

Abrisqueta, I., Abrisqueta, J. M., Tapia, L. M., Munguía, J. P., Conejero, W., Vera, J., Ruiz-Sánchez, M. C. (2013). "Basal crop coefficients for early-season peach trees." Agricultural Water Management, 121 (Apr): 158–163.

Adu, M.O., Yawson, D.O, Armah, F.A., Asare, P.A., Frimpong, K.A. (2018). Review: Meta-analysis of crop yiealds of full, deficit, and partial root-zone drying irrigation. Agricultural Water Management, 197: 79-90.

Adu, M.O., Yawson, D.O., Abano, E.E., Asare, P.A., Armah, F.A., Opoku, E.K. (2019). Does water-saving irrigation improve the quality of fruits and vegetables? Evidence from meta-analysis. Irrigation Science, 37, 669-690.

Agam, N., Evett, S. R., Tolk, J. A., Kustas, W. P., Colaizzi, P. D., Alfieri, J. G., McKee, L. G., Copeland, K. S., Howell, T. A., Chávez, J. L. (2012). Evaporative loss from irrigated interrows in a highly advective semiarid agricultural area. Advances in Water Resources, 50 (Dec): 20–30.

Aguado, A., Frías, J., García-Tejero, I., Romero, F., Muriel, J. L., Capote, N. (2012). Towards the Improvement of Fruit-Quality Parameters in Citrus under Deficit Irrigation Strategies. ISRN Agronomy.

Ahmad, P., Rasool, S., Gul, A., Sheikh, S.A., Akram, N.A., Ashraf, M., Kazi, A.M., Gucel, S. (2016). Jasmonates: Multifunctional Roles in Stress Tolerance. Frontiers in plant science, 7, 813.

Ahmadi, S.H., Andersen, M.N., Plauborg, F.,Poulsen, R.T., Jensen, C.R., Sepaskhah, A.R., Hansen, S. (2010). Effects of irrigation strategies and soils on field grown potatoes: Yield and water productivity. Agricultural Water Management, 97(11), 1923-1930.

Aiello, R., Bagarello, V., Barbagallo, S., Consoli, S., Di Prima, S., Giordano, G., Iovino, M. (2014). An assessment of the Beerkan method for determining the hydraulic properties of a sandy loam soil. *Geoderma*, *235*, 300–307.

Al Hagrey, S.A. (2007). Geophysical imaging of root-zone, trunk, and moisture heterogeneity. Journal of Experimental Botany, 58, 839-854.

Allen, R. G., Tasumi, M., Trezza, R. (2007). Satellite-based energy balance for mapping evapotranspiration with internalized calibration (METRIC)—Model. Journal of Irrigation and Drainage Engineering, 133 (4): 380–394.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M. (1998). Crop Evapotranspiration. Guidelines for Computing Crop Water Requirements; Irrigation and Drainage Paper No, 56; FAO: Rome, Italy, p. 300.

Allen, R.G., Pruitt, W.O., Wright, J.L., Howell, T.H., Ventura, F., Snyder, R., Itenfisu, D., Steduto, P., Berengena, J., Baselga Yrisarry, J., et al. (2006). A recommendation on standardized surface resistance for hourly calculation of reference ETo by the FAO56Penman-Monteith method. Agricultural Water Management, 81, 1–22.

Allred, B., Daniels, J.J., Reza Ehsani, M. (2008). Handbook of agricultural geophysics. CCR Press, Boca Raton, FL, 410.

Améglio, T., Archer, P., Cohen, M., Valancogne, C., Daudet, F.-a., Dayau, S., Cruiziat, P. (1999). Significance and limits in the use of predawn leaf water potential for tree irrigation. Plant Soil, 207, 155–167.

Anderson, R. G., J. G. Alfieri, R. Tirado-Corbalá, J. Gartung, L. G. McKee, J. H. Prueger, D. Wang, E. James, J. E. Ayars, and W. P. Kustas. (2017). “Assessing FAO-56 dual crop coefficients using eddy covariance flux partitioning.” Agricultural Water Management, 179 (Jan): 92–102.

Arbona, V., Iglesias, D.J., Jacas, J., Primo-Millo, E., Talon, M., Gomez-Cadenas, A. (2005). Hydrogel substrate amendment alleviates drought effects on young citrus plants. Plant Soil, 270, 73–82.

Aubinet, M., et al. (1999). Estimates of the annual net carbon and water exchange of forests: The EUROFLUX methodology. Advances in Ecological Research, 30: 113–175.

Aubinet, M., Grelle, A., Ibrom, A., Rannik, U., Moncrieff, J., Foken, T., Vesala, T. (2000). Estimates of the annual net carbon and water exchange of European forests: The EUROFLUX methodology. Advances in Ecological Research, 30, 113–175.

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A.; Norton, U., Ravetta, D.A., Schaeffer, S.M. (2004). Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, 141, 221–235.

Baldocchi, D., and Coauthors, 2001: FLUXNET: A new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities. Bulletin of the American Meteorological Society, 82, 2415–2434.

Baldocchi, D.D., Hincks, B.B., and Meyers, T.P. (1988). Measuring Biosphere-Atmosphere Exchanges of Biologically Related Gases with Micrometeorological Methods. Ecology, 69 (5), 1331-1340.

Ballester, C., Castel, J., Intrigliolo, D.S., Castel, J.R. (2013). Response of Navel Lane Latecitrus trees to regulated deficit irrigation: yield components and fruit composi-tion. Irrigation Science, 31, 333–341.

Ballester, C., Castel, J., Jiménez-Bello, M.A., Castel, J., Intrigliolo, D. (2013). Thermographic measurement of canopy temperature is a useful tool for predicting water deficit effects on fruit weight in citrus trees. Agricultural Water Management, 122, 1–6.

Ballester, C., Castela, J., Abd El-Mageedb, T.A., Castela, J.R., Intrigliolo, D. S. (2014). Long-term response of ‘Clementina de Nules’ citrus trees to summer regulated deficit irrigation. Agricultural Water Management,138, 78-84.

Ballester, C., J. Castel, L. Testi, D. S. Intrigliolo, and J. R. Castel. 2013. “Can heat-pulse sap flow measurements be used as continuous water stress indicators of citrus trees?” Irrigation Science, 31 (5): 1053–1063.

Ballester, C., Castel, J., Intrigliolo, D.S., Castel, J.R. (2011). Response of Clementina de Nules citrus trees to summer deficit irrigation. Yield components and fruit composition. Agricultural Water Management. 98, 1027-1032.

Bartels, D., Sunkar, R. (2005). Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 24, 23–58.

Bastiaanssen, W. G. M., Pelgrum, H., Wang, J., Ma, Y., Moreno, J. F., Roerink, G. J., van der Wal, T. (1998). A remote sensing surface energy balance algorithm for land (SEBAL): Part 2: Validation. Journal of Hydrology, 212 (Dec): 213–229.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205–207.

Behboudian, M.H., Singh, Z. (2001). Water relations and irrigation scheduling in grapevine. Horticultural Reviews, 27: 189-225.

Ben Hassine, A., Ghanem, M.E., Bouzid, S., Lutts, S. (2008). An inland and a coastal population of the Mediterranean xerohalophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycine betaine in response to salinity and water stress. Journal of Experimental Botany., 59, 1315–1326.

Beyá-Marshall, V., Herrera, J., Fichet, T., Trentacoste, E.R., Kremer, C. (2018). The effect of water status on productive and flowering variables in young ‘Arbequina’ olive trees under limited irrigation water availability in a semiarid region of Chile. Horticulture, Environment, and Biotechnology. 59(6), 815-826.

Binley, A. R3t Software Version 1.8 March 2013. Available online: http://www.es.lancs.ac.uk/people/amb/Freeware/R3t/R3t.htm (accessed on 17 October 2019).

Binley, A., Hubbard, S. S., Huisman, J. A., Revil, A., Robinson, D. A., Singha, K., Slater, L. D. (2015). The emergence of hydrogeophysics for improved understanding of subsurface processes over multiple scales. Water Resources Research, 51(6), 3837-3866

Bitella, G., Rossi, R., Loperte, A., Satriani, A., Lapenna, V., Perniola, M., & Amato, M. (2015). Geophysical techniques for plant, soil, and root research related to sustainability. In The Sustainability of Agro-Food and Natural Resource Systems in the Mediterranean Basin. Springer International Publishing, 353- 372.

Blackman, P. G., Davies, W. J. (1985). Root to shoot communication in maize plants of the effects of soil drying. Journal of Experimental Botany. 36 (1), 39-48.

Blum, A. (1996). Crop response to drought and the interpretation of adaptation. Journal of Plant Growth Regulation, 20,135–148.

Borken, W., Matzner, E. (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology, 15, 808–824.

Burba, G., Anderson, D. (2005). A brief practical guide to eddy covariance flux measurements: principles and workflow examples for scientific and industrial applications. Li-Cor Biosci.

Burba, G., and Anderson, D. (2010). A Brief Practical Guide to Eddy Covariance Flux Measurements: Principles and Workflow Examples for Scientific and Industrial Applications. LI-COR Biosciences, Lincoln, Nebraska, USA, 212.

Burgess, S. S. O., M. A. Adams, N. C. Turner, C. R. Beverly, C. K. Ong, A. A. H. Khan, and T. M. Bleby. 2001. “An improved heat pulse method to measure low and reverse rates of sap flow in woody plants.” Tree Physiology, 21 (9): 589–598.

Burt, C. M. and S. W. Styles. (2007). Drip and Micro Irrigation Design and Management for Trees, Vines, and Row Crops- Practice plus Theory. ITRC, Cal Poly, San Luis Obispo, California. 3rd Ed. 396.

Cammalleri, C., Rallo, G., Agnese, C., Ciraolo, G., Minacapilli, M., Provenzano, G. (2013). Combined use of eddy covariance and sap flow techniques for partition of ET fluxes and water stress assessment in an irrigated olive orchard. Agricultural Water Management, 120, 89-97.

Cancela, J. J., M. Fandino, B. J. Rey, and E. M. Martínez. (2015). ˜ Automatic irrigation system based on dual crop coefficient, soil and plant water status for Vitis vinifera (cv Godello and cv Mencía). Agricultural Water Management, 151 (Mar): 52–63.

Capra, A., Consoli, S., Russo, A., Scicolone, B. (2008). Integrated agro-economic approach to deficit irrigation on lettuce crops in Sicily (Italy). Journal of irrigation and drainage engineering. (ASCE) 134 (4), 437–445.

Capra, A., Consoli, S., Scicolone, B. (2013). Long-term climatic variability in Calabria and effects on drought and agrometeorological parameters. Water Resources Management, 27 (2): 601–617.

Carr, M.K.V. (2012). The water relations and irrigation requirements of citrus (Citrusspp.): a review. Experimental Agriculture. 48 (3), 347–377.

Casa, R., Russell, G., and Lo Cascio, B. (2000). Estimation of evapotranspiration from a field of linseed in central Italy. Agricultural and Forest Meteorology. 104 (4), 289-301.

Cassiani, G., Boaga, J., Vanella, D., Perri, M.T., Consoli, S., (2015). Monitoring and modelling of soil-plant root zone interaction: the joint use of ERT, sap flow and Eddy Covariance data to define the volume of an orange tree root zone. Hydrology and Earth System Sciences. 19, 2213.

Castel, J.R., Buj, A. (1990). Response of Salustiana orange to high frequency deficit irrigation. Irrigation Science. 11, 121-127.

Chai, Q., Gan, Y., Turner, N.C., Zhang, R.Z., Yang, C., Niu, Y., Siddique, K.H.M. (2014). Water-saving innovations in Chinese agriculture. Advances in Agronomy,126:147–197.

Chai, Q., Gan, Y., Zhao, C., Xu, H.L., Waskom, R.M., Niu, Y., Siddique, K.H.M. (2016). Regulated deficit irrigation for crop production under drought stress. A review. Agron. Sustain. Dev. Agronomy for sustainable development. 36.

Chalmers, D.J., Burge, G., Jerie, P.H., Mitchell, P.D. (1986). The mechanism of regulation of 'Bartlett' pear fruit and vegetative growth by irrigation withholding and regulated deficit irrigation. Journal of the American Society for Horticultural Science, 111(6), 904-907.

Chartzoulakis, K., Michelakis, N., Stefanoudaki, E. (1999). Water use, growth, yield and fruit quality of 'Bonanza' oranges under different soil water regimes. Advances in Horticultural Science, 13, 6-11.

Chiang, H. H., Dandekar, A. M. (1995). Regulation of proline accumulation in Arabidopsis during development and in response to dessication. Plant, Cell & Environment, *18*, 1280–1290.

Choné X., Van Leeuwen C., Dubourdieu D., Gaudillere J.P. (2001). Stem water potential is a sensitive indicator of grapevine water status. Annals of Botany, 87: 477- 483.

Ciaccia, C., La Torre, A., Ferlito, F., Testani, E., Battaglia, V., Salvati, L., Luca Salvati, Roccuzzo, G. (2019). Agroecological Practices and Agrobiodiversity: A Case Study on Organic Orange in Southern Italy. Agronomy, 9 (85), 2-13.

Cohen, A., Goell, A. (1988). Fruit growth and dry matter accumulation in grapefruit during periods of water withholding and after reirrigation. Australian Journal of Plant Physiology, 15, 633-639.

Cohen Y., Fuchs M., Green G. C., 1981. Improvement of the heat-pulse method for determining sap flow in trees. Plant, Cell & Environment, 4, 391-397.

Conesa, M.R., García-Salinas, M.D., De la Rosa, J.M., Fernandez-Trujillo, J.P., Domingo, R., Perez-Pastor, A. (2014). Effects of deficit irrigation applied during fruit growth period of late mandarin trees on harvest quality, cold storage and subsequent shelf-life. Journal Scientia Horticulturae, 165:344-351.

Consoli, S., & Papa, R. (2013). Corrected surface energy balance to measure and model the evapotranspiration of irrigated orange orchards in semi-arid Mediterranean conditions. Irrigation Science, 31(5), 1159-1171.

Consoli, S., Vanella, V. (2014). Mapping crop evapotranspiration by integrating vegetation indices into a soil water balance model. Agricultural Water Management, 143 (Sep): 71–81.

Consoli, S., Licciardello, F., Vanella, D., Pasotti, L., Villani, G., Tomei, F. (2016). Testing the water balance model criteria using TDR measurements, micrometeorological data and satellite-based information. Agricultural Water Management, 170 (May): 68–80.

Consoli, S., Stagno, F., Vanella, D., Boaga, J., Cassiani, G., Roccuzzo, G. (2017). Partial root-zone drying irrigation in orange orchards: Effects on water use and crop production characteristics. European Journal of Agronomy, 82 (Part A): 190–202.

Consoli, S., Stagno, F., Roccuzzo, G., Cirelli, G. L., Intrigliolo, F. (2014). Sustainable management of limited water resources in a young orange orchard. Agricultural Water Management, 132: 60–68.

Consoli, S., Inglese, G., Inglese, P. (2013b). Determination of evapotranspiration and annual biomass productivity of a cactus pear [Opuntia ficus-indica L. (Mill.)] orchard in a semiarid environment. Journal of Irrigation and Drainage Engineering, 139 (8): 680–690.

Consoli, S., Cirelli, G. L., Toscano, A. (2006). Monitoring crop coefficient of orange orchards using energy balance and the remote sensed NDVI. In Vol. 6359 of Remote sensing for agriculture, ecosystems, and hydrology VIII, 63590V. Bellingham, WA: SPIE.

Consoli, S., Facini, O., Nardino, M., Rossi, F. (2013a). Carbon balance and energy fluxes of a Mediterranean crop. Italian Journal of Agrometeorology, 19 (3): 15–24.

Consoli, S., O’Connell, N., & Snyder, R. (2006). Estimation of evapotranspiration of different-sized navel-orange tree orchards using energy balance. Journal of irrigation and drainage engineering, 132(1), 2-8.

Consoli, S., O’Connell, N., Snyder, R. (2006 a). Estimation of evapotranspiration of different-sized navel-orange tree orchards using energy balance. Journal of irrigation and drainage engineering, 132 (1), 2–8.

Consoli, S., O’Connell, N., Snyder, R. (2006 b). Measurement of light interception by navel orange orchard canopies: case study of Lindsay, California. Journal of irrigation and drainage engineering, 132 (1), 9-20.

Consoli, S., Intrigliolo, D.S., Stagno, F. (2012). Verso un uso più efficiente dell’irrigazione in agrumicoltura. Frutticoltura. 74, 66-72.

Corbari, C., Masseroni, D., and Mancini, M. (2012). Effetto delle correzioni dei dati misurati da stazioni eddy covariance sulla stima dei flussi evapotraspirativi. Italian Journal of Agrometeorology, 1, 35-51.

Corbari, C. (2010). Energy water balance and land surface temperature from satellite data for evapotranspiration control, PhD dissertation, Politecnico di Milano, Milan, Italy.

Cronje, P.J., Barry, G.H., Huysamer, M. (2013). Canopy position affects pigment expression and accumulation of flavedo carbohydrates of ‘Nules Clementine’mandarin fruit, thereby affecting rind condition. Journal of the American Society for Horticultural Science, 138 (3), 217-224.

Crowa, W. T., Kustas, W. P., Prueger, J. H. (2008). Monitoring root-zone soil moisture through the assimilation of a thermal remote sensingbased soil moisture proxy into a water balance model. Remote Sensing of Environment, 112 (4): 1268–1281.

D'Emilio, A., Aiello, R., Consoli, S., Vanella, D., Iovino, M. (2018). Artificial Neural Networks for Predicting the Water Retention Curve of Sicilian Agricultural Soils. Water, *10*, 1431.

Damour, G., Simonneau, T., Cochard, H., Urban, L. (2010) An overview of models of stomatal conductance at the leaf level. Plant, Cell & Environment, *33*, 1419–1438.

Davies, W.J., Wilkinson, S., Loveys, B. (2002). Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytologist, 153, 449–460.

Davies, W. J., Zhang, J. (1991). Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology, 42 55–76.

De la Hera, M.L., Romero, P., Gomez-Plaza, E., Martinez, A. (2007). Is partial root-zone drying an effective irrigation technique to improve water use efficiency and fruit quality in field-grown wine grapes under semiarid conditions? Agricultural Water Management, 87 (3), 261–274.

De Menezes de Assis Gomes, M., Magalhães Andrade Lagôa, A.M., Lázaro Medina, C., Caruso Machado, E., and Machado, M.A. (2004). Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange trees submitted to drought stress. Brazilian Journal of Plant Physiology, 16(3):155-161.

De Wit M., Stankiewicz, J. (2006). Changes in surface water supply across Africa with predicted climate change. Science, 31(311):1917–1921.

Detto, M., Montaldo, N., Albertson, J. D., Mancini, M., Katul, G. (2006). Soil moisture and vegetation controls on evapotranspiration in a heterogeneous Mediterranean ecosystem on Sardinia, Italy. Water Resources Research, 42 (8): 1–16.

Deuschle, K., Funck, D., Hellmann, H., Daschner, K., Binder, S., Frommer, W.B. (2001). A nuclear gene encoding mitochondrial Δ1-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. Plant Journal, 27, 345–356.

Dinga, R., Kanga, S., Zhang, Y., Haoa, X., Tonga, L., Dua, T. (2013). Partitioning evapotranspiration into soil evaporation and transpiration using a modified dual crop coefficient model in irrigated maize field with ground-mulching. Agricultural Water Management, 127 (Sep): 85–96.

Dodd, I.C., Egea, G., Davies, W.J. (2008). Abscisic acid signalling when soil moisture is heterogeneous: Decreased photoperiod sap flow from drying roots limits abscisic acid export to the shoots. Plant, Cell & Environment, 31, 1263–1274.

Dodd, I.C., Theobald, J.C., Bacon, M.A., Davies, W.J. (2006). Alternation of wet and dry sides during partial rootzone drying irrigation alters root-to-shoot signalling of ab- scisic acid. Functional Plant Biology, 33, 1081–1089.

Doorenbos, J., Kassam, A. (1979). Yield response to water. FAO Irrigation and Drainage Paper No 33. Rome, Italy, 193 pp.

Dragoni, D., Caylor, K.K., Schmid, H.P. (2009). Decoupling structural and environmental determinants of sap velocity. Part II. Observational application. Agricultural and Forest Meteorology, 149, 570–581.

Dry, P.R., Loveys, B.R., Düring, H. (1996). Effects of partial rootzone drying on grapevine vigour, yieald, composition of fruit and use of water. In: Stockley CS, Sas AN, Johnstone RS, Lee TH, editors. Proceedings of the 9th Australian Wine Industry Technical Conference. Adelaide, Australia, Winetitles, pp.126-131.

Dry, PR., Loveys BR, Düring H. (2000). Partial drying of the root-zone of grape. 2. Changes in the pattern of root development. Vitis, 39, 9–12.

Dry, PR., Loveys BR, McCarthy, M.G., Stoll, M. (2001). Strategic irrigation management in Australian vineyards. Journal International des Sciences de la Vigne et du Vin, 35:129-139.

Dry, PR., Loveys, BR. (1998). Factors influencing grapevine vigour and the potential for control with partial rootzone drying. Australian journal of grape and wine research, 4, 140-148.

Dry, P.R., Loveys, B.R. (2000). Partial drying of the rootzone of grape I. Transient changes in shoot growth and gas exchange. Vitis, 39, 3-8.

Du, T., Kang, S., Zhang, B., Li, S., Yang, X. (2008). Stem sap flow of grape under different drip irrigation patterns and its relationships with environmental factors in arid oasis region of Shiyang River basin. Chin. Journal of Applied Ecology, 19, 299–305.

Dzikiti, S., Steppe, K., & Lemeur, R. (2006). Partial rootzone drying of drip irrigated 'Navel'orange trees [Citrus sinensis (L.) Osbeck] under semi-arid tropical conditions. In V International Symposium on Irrigation of Horticultural Crops, 792 (pp. 249-256).

Eckman, R. M. 1994. "Influence of the sampling time on the kinematics of turbulent diffusion from a continuous source." Journal of Fluid Mechanics, 270: 349–375.

El-Otmani, M., Chouaibi, A., Azrof, C., Bouchaou, L., & Choukr-Allah, R. (2020). Response of Clementine Mandarin to Water-Saving Strategies under Water Scarcity Conditions. Water, 12(9), 2439.

Endo, T., Shimada, T., Nakata, Y., Fujii, H., Matsumoto, H., Nakajima, N., Ikoma, Y., Omura, M. (2018). Abscisic acid affects expression of citrus FT homologs upon floral induction by low temperature in Satsuma mandarin (Citrus unshiu Marc.). Tree Physiology, Volume 38 (5), 755–771.

English, M. J. (1990). Deficit irrigation: an analytical framework. Journal of irrigation and drainage engineering, 116, 399-412.

Er-Raki, S., Chehbouni, A., Hoedjes, J., Ezzahar, J., Duchemin, B., and Jacob, F. (2009). ”Citrus orchard evapotranspiration: Comparison between eddy covariance measurements and the FAO 56 approach estimates.” Plant Biosystems, 143 (1), 201-208.

Espadafor, M., Orgaz, F., Testi, L., Lorite, I. J., González-Dugo, V., Fereres, E. (2017). Responses of transpiration and transpiration efficiency of almond trees to moderate water deficits. Scientia Horticulturae, 225, 6–14.

FAO (Food and Agriculture Organization). 2003. “FAO Statistics.” Accessed March 15, 2019.

Farihi, J., Gänsicke, BT., Koester, D. (2013). Evidence for water in the rocky debris of a disrupted extrasolar minor planet. Science, 342:218–220.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: Effects, mechanisms and management. In Sustainable Agriculture; Springer: Dordrecht, The Netherlands, pp. 153–188.

Faurès, J. M., D. Bartley, M. Bazza, J. Burke, J. Hoogeveen, D. Soto, and P. Steduto. 2013. Climate smart agriculture sourcebook. Rome: Food and Agriculture Organization.

Fereres, E. & Soriano, M.A. (2006). Deficit irrigation for reducing agricultural water use. Journal of Experimental Botany, 58(2), pp.147–159.

Fernández, J. E., Alcon, F., Diaz-Espejo, A., Hernandez-Santana, V., Cuevas, M.V. (2020). Water use indicators and economic analysis for on-farm irrigation decision: A case study of a super high density olive tree orchard. Agricultural Water Management, 237, 106074.

Fernández, J. E., Díaz-Espejo, A., Infante, J. M., Durán, P. Palomo, M. J., Chamorro, V. Girón, I. F., Villagarcía, L. (2006). Water relations and gas exchange in olive trees under regulated deficit irrigation and partial rootzone drying. Plant and Soil, 284:273-291.

Fernández-Trujillo, J.P., Obando, J., Martinez, J.A., Alarcón, A.L., Eduardo, I., Arús, P., Monforte, A.J. (2007). Mapping fruit susceptibility to posharvest physiological disorders and decay using a collection of near-isogenic lines of melon. Journal of the American Society for Horticultural Science, 132, 739–748

Ferreira, M. I., J. Silvestre, N. Conceicao, and A. C. Malheiro. 2012. “Crop and stress coefficients in rainfed and deficit irrigation vineyards using sap flow techniques. Irrigation Science, 30 (5), 433–447.

Finkelstein, R. (2013). Abscisic acid synthesis and response. In *The Arabidopsis Book*; American Society of Plant Biologists: Rockville, MR, USA, p. 111.

Foken, T., (2008). The energy balance closure problem - An overview. Ecological Applications, 18, 1351–1367.

Forouzani, M., Karami, E. (2011). Agricultural water poverty index and sustainability. Agronomy for Sustainable Development, 31, 415-432.

Fracasso, A., Telò, L., Lanfranco, L., Bonfante, P., , Amaducci, S. (2020). Physiological Beneficial Effect of *Rhizophagus intraradices* Inoculation on Tomato Plant Yield under Water Deficit Conditions. Agronomy, 10, 71.

Galindo, A., Collado-González, J., Griñán, I., Corell, M., Centeno, A., Martín-Palomo, M. J., Girón, I. F., Rodríguez, P., Cruz, Z. N., Memmi, H., Carbonell-Barrachina, A. A., Hernández, F., Torrecillas, A., Moriana, A., López-Pérez, D. (2018). Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. Agricultural Water Management, 202, 311-324.

Gan, Y., Siddique, K.H.M., Turner, N.C., Li, X-G., Niu, J-Y., Yang, C., Liu, L., Chai, Q. (2013). Ridge-furrow mulching systems—an innovative technique for boosting crop productivity in semiarid rain-fed environments. Advances in Agronomy, 118, 429-476.

García-Orellana, Y., Ortuño, M.F., Conejero, W., Ruiz-Sánchez, M.C. (2013). Diurnal variations in water relations of deficit irrigated lemon trees during fruit growth period Span. Journal of Agricultural Research. 11, 137-145.

García-Tejero, I., Durán-Zuazo, V.H., Jiménez-Bocanegra, J.A., Muriel-Fernández, J.L. (2011). Improved water-use efficiency by deficit-irrigation programmes: Implications for saving water in citrus orchards. Scientia horticulturae. 128 (3), 274-282.

García-Tejero, I., Durán-Zuazo, V.H., Muriel-Fernández, J.L., Jiménez-Bocanegra, J.A. (2011). Linking canopy temperature and trunk diameter fluctuations with other physiological water status tools for water stress management in citrus orchards. Functional Plant Biology, 38, 106–117.

García-Tejero, I., Romero-Vicente, R., Jiménez-Bocanegra, J.A., Martínez-García, G., Durán- Zuazo, V.H., Muriel-Fernández, J.L. (2010). Response of citrus trees to deficit irrigation during different phenological periods in relation to yield, fruit quality, and water productivity. Agricultural Water Management, 97, 689-699.

Gasque, M., Martí, P., Granero, B., González-Altozano, P. (2016). Effects of long-term summer deficit irrigation on 'Navelina' citrus trees. Agricultural Water Management, 169, 140-147.

Giannico, V., Chen, J., Shao, C., Ouyang, Z., John, R., Lafortezza, R. (2018). Contributions of landscape heterogeneity within the footprint of eddy-covariance towers to flux measurements." Agricultural and Forest Meteorology, 260–261 (Oct): 144–153.

Giardini L. (1995). Agronomia generale. Patron Editore, Padova.

Ginestar, C., Castel, J.R. (1996). Responses of young clementine citrus trees to water stress during different phenological periods. Journal of Horticultural Science, 71, 551-559.

Girona, J. (2002). Regulated deficit irrigation in peach. A global analysis. Acta Horticulturae, 592, 335–342.

Girona, J., M. Mata and J. Marsal. (2005). Regulated deficit irrigation during the kernel-filling period and optimal irrigation rates in almond. Agricultural water management, 43,75–98.

Girona, J., Marsal, J., López, G. (2006b). Establishment of stem water potential thresholds for the response of ‘O’Henry’ peach fruit growth to water stress during stage III of fruit growth. Acta Horticulturae. 713, 197-201.

Girona, J., Mata, M., Del Campo, J., Arbonés, A., Bartra, E., Marsal, J. (2006a). The use of midday leaf water potential for scheduling deficit irrigation in vineyards. Irrigation Science, 24,115-127.

Girona, J., Mata, M., Fereres, E., Goldhamer, D.A., Cohen, M. (2002). Evapotranspiration and soil water dynamics of peach trees under water deficits. Agricultural Water Management, 54(2), 107-122.

Gleick, PH., Palaniappan, M. (2010). Peak water limits to freshwater withdrawal and use. Proceedings of the National Academy of Sciences, 107:11155–11162.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. Science, 327:812–818.

Goldhamer, D. A., Fereres, E., Mata, M., Girona, J., Cohen, M. (1999), Sensitivity of Continuous and Discrete Plant and Soil Water Status Monitoring in Peach Trees Subjected to Deficit Irrigation. American Society for Horticultural Science, 124, 437-444.

Goldhammer, D.A., Salinas, M., Crisosto, C., Day, K.R., Soler Moriana, M.A. (2001). Effects of regulated deficit irrigation and partial root zone drying on late harvest peach tree performance. V International Peach Symposium, 592, 343–350.

Gomez-Cadenas, A., Tadeo, F.R., Primo-Millo, E., Talon, M. (1998). Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. Plant Physiology, 103, 475–484.

Gonzalez-Altozano, P, Castel, J.R. (1999). Regulated deficit irrigation in ‘Clementina de Nules’ citrus trees. I. Yield and fruit quality effects. Journal of Horticultural Science and Biotechnology, 74(6), 706–713.

Gonzalez-Altozano, P. and J.R. Castel. (2003). Riego deficitario controlado en ‘Clementina de Nules’. II. Efecto sobre el crecimiento vegetativo. Spanish journal of agricultural research, 1:93–101.

Gonzalez-Altozano, P., Castel, J.R. (2000). Regulated deficit irrigation in ‘Clementina de Nules’ citrus trees. II: Vegetative growth. Journal of Horticultural Science and Biotechnology, 75:388–392

González-Altozano, P., Pavel, E.W., Oncins, J.A., Doltra, J., Cohen, M., Paço, T., Massai, R., Castel, J.R. (2008). Comparative assessment of five methods of determining sap flow in peach trees. Agricultural Water Management, 95, 503-515.

Gonzalez-Dugo, V., Ruz, C., Testi, L., Orgaz, F., Fereres, E. (2018). The impact of deficit irrigation on transpiration and yield of mandarin and late oranges. Irrigation Science, 36, 227–239.

Gotur, M., Sharma, D.K., Joshi, C.J., Rajan, R. (2018). Partial root-zone drying technique in fruit crops: A review paper. International Journal of Chemical Studies, 6, 900-903.

Granier, A., Biron, P., Lemoine, D. (2000). Water balance, transpiration and canopy conductance in two beech stands. Agricultural and Forest Meteorology, 100, 291—308.

Green S., (2009). Measurements of Sap Flow by the Heat-Pulse Method. An instruction manual for the HPV system. New Zeland.

Green, S. (1998). Measurements of sap flow by the heat-pulse method. An instruction manual for the HPV system. Palmerston North, New Zealand: Hort Research Institute.

Green, S.R., Clothier, B., Jardine, B. (2003). Theory and practical application of heat pulse to measure sap flow. Agronomy Journal, 95, 1371–1379.

Grilo, F.S., Scalisi, A., Pernice, F., Morandi, B., Lo Bianco, R. (2019). Recurrent deficit irrigation and fruit harvest affect tree water relations and fruitlet growth in 'Valencia' orange. European Journal of Horticultural Science, 84, 177-187.

Guo, Y.-P., Zhou, H.-F. & Zhang, L.-C. (2006). Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. Scientia Horticulturae, 108(3), pp.260–267.

Hanson, A.D., Rathinasabapathi, B., Rivoal, J., Burnet, M., Dillon, M.O., Gage, D.A. (1994). Osmoprotective compounds in the Plumbaginaceae: A natural experiment in metabolic engineering of stress tolerance. Proceedings of the National Academy, USA, *91*, 306–310.

Hanson, B., Schwankl, L., Grattan, S. R., Prichard, T. (1997). Drip irrigation for row crops. Cooperative Extension, Department of Land, Air, and Water Resources, Univ. of Calif., Davis, California, 238.

Hartung, A., Sauter, E. (2002). Hose Abscisic acid in the xylem: Where does it come from, where does it go? Journal of Experimental Botany, 53, 27–32.

Hayano-Kanashiro, C., Calderon-Vazquez, C., Ibarra-Laclette, E., Herrera-Estrella, L., Simpson, J. (2009). Analysis of gene expression and physiological responses in three mexican maize landraces under drought stress and recovery irrigation. *PLoS ONE*, *4*, e7531.

Heilman, J. L., Ham, J. M. (1990). Measurement of mass flow rate of sap in Ligustrum japonicum. Horticultural Science, 25 (4): 465–467.

Heydari, N. (2014). Water productivity in agriculture: challenges in concepts, terms and values. Irrigation and Drainage, 63, 22–28.

Hong, Z., Lakkineni, K., Zhang, Z., Verma, D.P.S. (2000). Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiology, *122*, 1129–1136.

Hsiao, T.C. (1973). Plant Responses to Water Stress. Annual Review of Plant Physiology, 24(1), pp.519–570.

Hsieh, C.-I., G. Katul, and T.-W. Chi. 2000. “An approximate analytical model for footprint estimation of scalar fluxes in thermally stratified atmospheric flows.” Advances in Water Resources, 23 (7): 765–772.

Hubick, K.T., Reid, D.M. (1980). A rapid method for the extraction and analysis of abscisic acid from plant tissue. Plant Physiology, 65, 523–525.

Hueso, J.J., Cuevas, J. (2010). Ten consecutive years of regulated deficit irrigation probe the sustainability and profitability of this water saving strategy in loquat. Agricultural Water Management, 97 (5), 645-650.

Hussain, S., Khalid, M.F., Saqib, M., Ahmad, S., Zafar, W., Rao, M.J., Morillon, R., Anjum, M.A. (2018). Drought tolerance in citrus rootstocks is associated with better antioxidant defense mechanism. Acta Physiologiae Plantarum, 40, 135.

Hutton, R.J., Landsberg, J.J., Sutton, B.G. (2007). Timing irrigation to suit citrus phenology: a means to reduce water use without compromising fruit yield and quality? Australian Journal of Experimental Agriculture, 47, 71-80.

Hutton, R.J., Loveys, B.R. (2011). A partial root zone drying irrigation strategy for citrus-effects on water use efficiency and fruit characteristics. Agricultural Water Management, 98:1485–1496.

Incrocci, L., Riccò, E. (2004). Impianti per l’irrigazione e per la fertirrigazione. QUADERNO ARSIA 5/2004.

Intergovernmental Panel on Climate Change, 2014. Climate Change 2014 Mitigation of Climate Change.

Intrigliolo, D.S., Ballester, C., Castel, J.R. (2013). Carry-over effects of deficit irrigation applied over seven seasons in a developing Japanese plum orchard. Agricultural Water Management, 128, 13-18.

Intrigliolo, D.S., Castel, J.R. (2009). Response of Vitis vinifera cv 'Tempranillo' to partial rootzone drying in the field: water relations, growth, yield and fruit and wine quality. Agricultural Water Management, 96, 282–292.

Jamshidi, S., Zand-Parsab, S., Kamgar-Haghighi, A. A., Shahsavarc, A. R., Niyogia, D. (2020). Evapotranspiration, crop coefficients, and physiological responses of citrus trees in semi-arid climatic conditions. Agricultural Water Management, 227, 105838.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Renmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncriff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R. (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology, 7:269–278.

Johnson, R.S., Handley, D.F., DeJong, T.M. (1992). Long-term response of early maturing peach trees to postharvest water deficit. Science Journal of the American Society for Horticultural Science, 117, 881-886

Jones, H.G. (2004). Irrigation Scheduling: advantages and pitfalls of plant based methods. Journal of Experimental Botany, 55, 2427–2436.

Jones, H.G. (1992). Plants and microclimate. A quantitative approach to environmental plant physiology. 2nd edition. Cambridge: Cambridge University Press.

Jovanovic, Z., Radmila Stikic, R. (2018). Partial Root-Zone Drying Technique: from Water Saving to the Improvement of a Fruit Quality. Frontiers in Sustainable Food Systems. 1, 3.

Kang, S., Hu, X., Goodwin, I., Jerie, P. (2002) Soil water distribution, water use, and yield response to partial root zone drying under a shallow groundwater table condition in a pear orchard. Scientia Horticulturae, 92:277– 291.

Kang, S., Hu, X., Jerie, P., Zhang, J. (2003). The effects of partial rootzone drying on root, trunk sap flow and water balance in an irrigated pear (Pyrus communis L.) orchard. Journal of Hydrology, 280, 192–206.

Kang, S., Shi, W., Zhang, J. (2000). An improved water-use efficiency for maize grown under regulated deficit irrigation. Field Crops Research, 67: 207–214.

Keller, J. and Karmeli, D. (1975) Trickle Irrigation Design Parameters. Rain Bird Sprinkler Manufacturing Corporation, Glendora, 133p.

Keller, J., Karmeli, D. (1974). Trickle irrigation design parameters. Transactions of the ASAE, 17(4), 678–684.

Khedr, A.H., Abbas, M.A., Wahid, A.A., Quick, W.P., Abogadallah, G.M. (2003). Proline induces the expression of salt-stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. Journal of Experimental Botany, 54, 2553–2562.

Köhler, M., Schwendenmann, L., Hölscher, D. (2010). Throughfall reduction in a cacao agroforest: tree water use and soil water budgeting. Agricultural and Forest Meteorology, 150, 1079–1089.

Küppers, M., O'Rourke, L., Bockelée-Morvan, D., Zakharov, V., Lee, S., Von Allmen, P., Carry, B., Teyssier, D., Marston, A., Müller, T., Crovisier, J., Barucci, M.A., Moreno, R., (2014). Localized sources of water vapour on the dwarf planet (1) Ceres. Nature, 505:525–527.

Kusakabe, A., Contreras-Barragan, B.A., Simpson, C.R., Enciso, J.M., Nelson, S.D., Melgar, J.C. (2016). Application of partial rootzone drying to improve irrigation water use efficiency in grapefruit trees. Agricultural Water Management, 178, 66-75

L ´opez-L ´opez, M., Espadafor, M., Testi, L., Lorite, I. J., Orgaz, F., Fereres, E. (2018). Water use of irrigated almond trees when subjected to water deficits. Agricultural Water Management, 195 (Jan): 84–93.

Lal, R., Delgado, J. A., Groffman, P. M., Millar, N., Dell, C., & Rotz, A. (2011). Management to mitigate and adapt to climate change. Journal of Soil and Water Conservation. 66 (4), pp.276–285.

Lamm, F. R. (2009). Unique challenges with subsurface drip irrigation. ASABE Paper No. 095927. St. Joseph, Mich.: ASABE.

Lamm, F. R. and C. R. Camp. (2007). Subsurface drip irrigation. Chapter 13 in Microirrigation for Crop Production - Design, Operation and Management, Elsevier, Amsterdam, 473-551.

Lamm, F. R., & Rogers, D. H. (2017). Longevity and performance of a subsurface drip irrigation system. Transactions of the ASABE, 60(3), 931-939.

Lamm, F. R., Bordovsky, J. P., Schwankl, L. J., Grabow, G. L., Enciso-Medina, J., Peters, R. T., Colaizzi, P. D., Trooien, T. P., Porter, D. O. (2012). Subsurface drip irrigation: Status of the technology in 2010. Transactions of the ASABE, 55 (2), 483-491.

Lamm, F. R., O'Brien, D. M., & Rogers, D. H. (2015). Economic comparison of subsurface drip and center-pivot sprinkler irrigation using spreadsheet software. Applied Engineering in Agriculture, 31(6), 929-936.

Lee, X., W. Massman, and B. Law, eds. 2004. Vol. 29 of Handbook of micrometeorology: A guide for surface flux measurement and analysis. New York: Springer.

Li, Q., Song, P., Zhou, B., Xiao, Y., Muhammad, T., Liu, Z., Zhou, H., Li, Y. (2019). Mechanism of intermittent fluctuated water pressure on emitter clogging substances formation in drip irrigation system utilizing high sediment water. Agricultural Water Management, 215, 16-24.

Liang, J., Zhang, J., Wong, M.H. (1997). How do roots control xylem sap ABA concentration in response to soil drying? Plant and Cell Physiology, 38,10-16

Liu, F., Shahnazari, A., Andersen, M.N., Jacobsen, S.E., Jensen, C.R. (2006). Effects of deficit irrigation (DI) and partial root drying (PRD) on gas exchange, biomass partitioning, and water use efficiency in potato. Scientia Horticulturae. 109, 113-117

Liu, X., Kang, S., Li, F. (2009). Simulation of artificial neural network model for trunk sap flow of Pyrus pyrifolia and its comparison with multiple-linear regression. Agricultural Water Management, 96, 939–945.

Longo-Minnolo, G., Vanella, D., Consoli, S., Intrigliolo, D.S., Ramírez-Cuesta, J.M. (2020). Integrating forecast meteorological data into the ArcDualKc model for estimating spatially distributed evapotranspiration rates of a citrus orchard. Agricultural Water Management, 231, 105967.

Lopez-Bernal, A., Garcia-Tejera, O., Vega, V. A., Hidalgo, J. C., Testi, L., Orgaz, F., Villalobos, F. J. (2015). "Using sap flow measurements to estimate net assimilation in olive trees under different irrigation regimes." Irrigation Science, 33 (5): 357–366.

Loutfy, N., El-Tayeb, M.A., Hassanen, A.M., Moustafa, M.F., Sakuma, Y., Inouhe, M. (2012). Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (Triticum aestivum). Journal of Plant Research, 125, 173–184.

Loveys, B.R., Stoll, M., Dry, P.R. and McCarthy, M.G. (2000). Using plant physiology to improve the water use efficiency of horticultural crops. Acta Horticulturae, 537, 187-197.

Loveys, B.R., Grant, J., Dry, P.R., McCarthy, M.G. (1997). Progress in the development of partial rootzone drying. Aust. Grapegrow. Winemak., 403, 18-20.

López-López, M., Espadafor, M., Testi, L., Lorite, I. J., Orgaz, F., Fereres, E. (2018). Water use of irrigated almond trees when subjected to water deficits. Agricultural Water Management, 195, 84–93.

Maestre-Valero, J. F., Testi, L., Jiménez-Bello, M. A., Castel, J. R., and Intrigliolo, D. S. (2017). Evapotranspiration and carbon exchange in a citrus orchard using eddy covariance. Irrigation Science, 35, 397–408.

Maranon-Jiménez, S., J. Van den Bulcke, A. Piayda, J. Van Acker, M. Cuntz, C. Rebmann, Steppe, K. (2017). X-ray computed microtomography characterizes the wound effect that causes sap flow underestimation by thermal dissipation sensors. Tree Physiology, 38 (2), 287–301.

Marsal, J., Mata, M., Del Campo, J., Arbones, A., Vallverdú, X., Girona.J., Olivo, N. (2008). Evaluation of partial root-zone drying for potential eld use as a deficit irrigation technique in commercial vineyards according to two different pipeline layouts. Irrigation Science, 26, 347–356.

Marshall, D.C. (1958). Measurement of sap flow in conifers by heat transport. Plant Physiology, 33, 385-396

Martínez-Gimeno, M.A., Bonet, L., Provenzano, G., Badal, E., Intrigliolo, D.S. and Ballester C. (2018). Assessment of yield and water use efficiency of Clementine trees under surface and subsurface drip irrigation. Agricultural Water Management, 206.

Mary, B., Vanella, D., Consoli, S., Cassiani, G. (2019). Assessing the extent of citrus trees root apparatus under defcit irrigation via multi-method geo-electrical imaging. Scientific Reports, 9, 1-10.

Massman, W.J., Lee, X. (2002). Eddy covariance flux corrections and uncertainties in long-term studies of carbon and energy exchanges Agricultural and Forest Meteorology, 113 (2002), pp. 121-144

Mattioli, R., Costantino, P., Trovato, M. (2009). Proline accumulation in plants. Not only stress. Plant Signaling & Behavior, 4, 1016–1018.

Mehanni, AH., and Chalmers, D.J. (1986). Effect of irrigation with saline water on soil properties and salinization of perennial pasture soils. Australian Journal of Experimental Agriculture, 26(2), 173-179.

Meyers, T., Hollinger, S. (2004). An assessment of storage terms in the surface energy balance of maize and soybean. Agricultural and Forest Meteorology, 125, 105-115.

Melgar, J. C., Dunlop, J. M., Syvertsen, J. P. (2010). Growth and physiological responses of the citrus rootstock Swingle citrumelo seedlings to partial rootzone drying and deficit irrigation. Journal of Agricultural Science. 148:593-602.

Michot, D., Benderitter, Y., Dorigny, A., Nicoullaud, B., King, D., Tabbagh, A. (2003). Spatial and temporal monitoring of soil water content with an irrigated corn crop cover using surface electrical resistivity tomography. Water Resources Research, 39, 1138.

Milborrow B.V. (2001). The pathway of biosynthesis of abscisic acid in vascular plants: a review of the present state of knowledge of ABA biosynthesis. Journal of Experimental Botany, 52, 1145–1164.

Mingo, D.M., Theobald, J.C., Bacon, M.A., Davies, W.J., Dodd, I.C. (2004). Biomass allo- cation in tomato (Lycopersicon esculentum) plants grown under partial rootzone drying: enhancement of root growth. Functional Plant Biology. 31, 971–978.

Mitchell, P.D., Chalmers, D.J., Jerie, P.H., Burge, G., (1986). The use of initial with- holding of irrigation and tree spacing to enhance the effect of regulated deficit irrigation on pear trees. Journal of the American Society for Horticultural Science, 111, 858–861.

Mitchell, P.D., Jerie, P.H., Chalmers, D.J. (1984). The effects of regulated water deficits on pear tree growth, flowering, fruit growth and yield. Journal of the American Society for Horticultural Science, 109, 604–606.

Mossad, A., Farina, V., Lo Bianco, R. (2020). Fruit Yield and Quality of 'Valencia' Orange Trees under Long-Term Partial Rootzone Drying. Agronomy, 10, 164.

Mossad, A., Scalisi, A., Lo Bianco, R. (2018). Growth and water relations of field-grown 'Valencia' orange trees under long-term partial rootzone drying. Irrigation Science, 36, 9–24.

Motisi, A., Consoli, S., Papa, R., Cammalleri, C., Rossi, F., Minacapilli, M., and Rallo, G. (2012). Eddy covariance and sap flow measurement of energy and mass exchanges of woody crops in a Mediterranean environment. Acta Horticulturae, 951, 121-128.

Mougheith, M.J., El-Ashram, M., Amerhom, G., Madbouly, W. (1977). Effect of different rates of irrigation on Navel oranges trees. II. Yield and fruit quality. Annals of Agricultural Science, Moshtohor. 8, 119-127.

Moustakas, M., Sperdouli, I., Kouna, T., Chrysovalantou-Irene Antonopoulou, C-I., Therios, I. (2011). Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of Arabidopsis thaliana leaves. Plant Growth Regulation, 65, 315-325.

Mugnai, S. (2004). Elementi di ecofisiologia vegetale. Uso razionale delle risorse nel florovivaismo: l’acqua. Quaderno ARSIA. 5.

Naor, A. (2006). Irrigation scheduling and evaluation of tree water status in deciduous orchards. Horticultural Reviews, 32, 111-166.

Naser, L., Kourosh, V., Bahman, K., Reza, A. (2010). Soluble sugars and proline accumulation play a role as effective indices for drought tolerance screening in Persian walnut (Juglans regia L.) during germination. Fruits, 65(2), 97-112.

Navarro, J.M., Botia, P., Pérez-Pérez, J.G. (2015). Influence of deficit irrigation timing on the fruit quality of grapefruit (Citrus paradise Mac.). Food Chemistry, 175, 329-336.

Navarro, J.M., Pérez-Pérez, J.G, Romero, P., Botia, P. (2010). Analysis of the changes in quality in mandarin fruit, produced by deficit irrigation treatments. Food Chemistry, 119, 1591-1596.

Noy-Meir, I., 1973. Desert ecosystems: environment and producers. Annual Review of Ecology and Systematics, 4, 51-58.

Norman, J. M., Kustas, W. P., & Humes, K. S. (1995). Source approach for estimating soil and vegetation energy fluxes in observations of directional radiometric surface temperature. Agricultural & Forest Meteorology, 77(3), 263-293.

Novick, K., S. Brantley, C. Ford Miniat, J. Walker, and J. M. Vose. 2014. "Inferring the contribution of advection to total ecosystem scalar fluxes over a tall forest in complex terrain." Agricultural and Forest Meteorology, 185 (Feb): 1–13.

O'Connell, M. G., and I. Goodwin. 2007. "Responses of 'Pink Lady' apple to deficit irrigation and partial rootzone drying: Physiology, growth, yield, and fruit quality." Australian Journal of Agricultural Research, 58 (11): 1068–1076.

Odhiambo, L. O., and S. Irmak. 2012. "Evaluation of the impact of surface residue cover on single and dual crop coefficient for estimating soybean actual evapotranspiration." Agricultural Water Management, 104 (Feb): 221–234.

Oishi, A.C., Oren, R., and Stoy, P.C. (2008). Estimating components of forest evapotranspiration: A footprint approach for scaling sap flux measurements. Agricultural and forest meteorology, 148 (11), 1719-1732.

Ortuño, M.F., Alarcón, J. J., Nicolás, E., Torrecillas, A., (2004 a). Comparison of continuously recorded plant-based water stress indicators for young lemon trees. Plant Soil, 267, 263–270.

Ortuño, M.F., Alarcón, J.J., Nicolás, E., Torrecillas, A. (2004b). Interpreting trunk diameter changes in young lemon trees under deficit irrigation. Plant Science, 227, 275–280.

Ortuño, M.F., Conejero, W., Moreno, F., Moriana, A., Intrigliolo, D.S., Biel, C., Mellisho, C.D., Pérez-Pastor, A., Domingo, R., Ruiz-Sánchez, M.C., Casadesus, J., Bonany, J., Torrecillas, A. (2010). Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. Agricultural Water Management, 97, 1–11.

Ortuño, M.F., García-Orellana, Y., Conejero, W., Ruiz-Sánchez, M.C., Alarcón, J.J., Torrecillas, A. (2006). Stem and leaf water potentials, gas exchange, sap flow and trunk diameter fluctuations for detecting water stress in lemon trees. Trees, 20, 1–8.

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.S. (2014). Response of plants to water stress. Frontiers in Plant Science, 13;5:86.

Paco, T.A., et al., 2009. Evapotranspiration from a Mediterranean evergreen oak savannah: the role of trees and pasture. J. Hydrol. 369 (1–2), 98–106.

Paço, T. A., I. Pôças, M. Cunha, J. C. Silvestre, F. L. Santos, P. Paredes, and L. S. Pereira. 2014. “Evapotranspiration and crop coefficients for a super intensive olive orchard. An application of SIMDualKc and METRIC models using ground and satellite observations.” Journal of Hydrology, 519: 2067–2080.

Paço, T. A., Ferreira, M. I., Conceicao., N. (2006). Peach orchard evapotranspiration in a sandy soil: Comparison between eddy covariance measurements and estimates by the FAO 56 approach. Agricultural Water Management, 85 (3): 305–313.

Paço, T., Ferreira, M., Rosa, R., Paredes, P., Rodrigues, G., Conceição, N., Pacheco, C., Pereira, L. (2012). The dual crop coefficient approach using a density factor to simulate the evapotranspiration of a peach orchard: SIMDualKc model versus eddy covariance measurements. Irrigation Science, 30 (2): 115–126.

Page, A.L., Miller, R.H., Keeney, D.R. (1982). *Methods of Soil Analysis. Part 2. Chemical and Microbial Properties*; American Society of Agronomy No. 9 (Part 2) in the Agronomy Series; ASA/SSSA: Madison, WI, USA.

Pappalardo, H., Nicolosi, E., Vanella, D., Torrisi, B., Allegra, M., Stagno, F., Saitta, D., Consoli, S., Roccuzzo, G., Ferlito, F. (2019). Risposta fisiologica di un agrumeto allo stress idrico controllato. Frutticoltura. n.1.

Parida, A.K., Dagaonkar, V.S., Phalak, M.S., Aurangabadkar, L.P. (2008). Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. Acta Physiologiae Plantarum, 30, 619–627.

Parvizi, H., Sepaskhah, A.R., Ahmadi, S.H. (2014). Effect of drip irrigation andfertilizer regimes on fruit yields and water productivity of a pomegranate (Punica granatum (L.) cv. Rabab) orchard. Agricultural Water Management, 146, 45–56.

Parvizi, H., Sepaskhah, A.R., Ahmadi, S.H. (2016). Physiological and growth responsesof pomegranate tree (Punica granatum (L.) cv. Rabab) under partial root zonedrying and deficit irrigation regimes. Agricultural Water Management, 163, 146–158.

Patakas, A., Noitsakis, B., Chouzouri, A. (2005). Optimizations of irrigation water use in grapevines using the relationship between transpiration and plant water status. Agriculture, Ecosystems & Environment. 106:253–259.

Peng, Y.H., Rabe, E. (1998). Effect of differing irrigation regimes on fruit quality, yield, fruit size and net CO_2 assimilation of ‘Mihowase’ satsuma. Journal of Horticultural Science and Biotechnology, 73, 229-234.

Pérez-Pérez, J. G., Robles, J. M., Botía, P. (2009). Influence of deficit irrigation in phase III of fruit growth on fruit quality in ‘lane late’ sweet orange. Agricultural Water Management. 96, 969–974.

Pérez-Pérez, J. G.,García, J.,Robles, J. M.,Botía, P. (2010). Economic analysis of navel orange cv. 'Lane late' grown on two different drought-tolerant rootstocks under deficit irrigation in South-eastern Spain. Agricultural Water Management, 97,157-164.

Pérez-Pérez, J.G., Dodd, I.C. (2015). Sap fluxes from different parts of the rootzone modulate xylem ABA concentration during partial rootzone drying and re-wetting. Journal of Experimental Botany, 66, 2315–2324.

Pérez-Pérez, J.G., Navarro, J.M., Robles, J.M., Dodd, I.C. (2018). Prolonged drying cycles stimulate ABA accumulation in Citrus macrophylla seedlings exposed to partial rootzone drying. Agricultural Water Management, 210, 271-278.

Pérez-Pérez, J., Romero, P., Navarro, J., Botía, P. (2008). Response of sweet orange cv. 'lane late' to deficit irrigation strategy in two rootstocks. II: Flowering, fruit growth, yield and fruit quality. Irrigation Science, 26, 519-529.

Perez-Priego, O., T. S. El-Madany, M. Migliavacca, A. S. Kowalski, M. Jung, A. Carrara, O. Kolle, M. Pilar Martin, J. Pacheco-Labrador, G. Moreno, and M. Reichstein. 2017. Evaluation of eddy covariance latent heat fluxes with independent lysimeter and sapflow estimates in a Mediterranean savannah ecosystem. Agricultural and Forest Meteorology, 236 (Apr): 87–99.

Perrier, A., Alves, I., Walter, I., Elliott, R. (2006). A recommendation on standardized surface resistance for hourly calculation of reference ETo by the FAO56 Penman-Monteith method. Agricultural Water Management, 81, 1–22.

Phene, C. J. (1996). Shovel versus computer. Irrigation Business and Technology, 4(3),6.

Phene, C.J., (1995). The sustainability and potential of subsurface drip irrigation. In Proceedings of the Fifth

International Micro Irrigation Congress, Orlando, Florida. 359-367.

Phene, C.J., Davis, K.R., Hutmacher, R.B., McCormick, R.L. (1986). Advantages of subsurface irrigation for processing tomatoes. In II International Symposium on Processing Tomatoes, XXII IHC 200, 101-114.

Phene, C.J., Hutmacher, R.B., Ayars, J.E. (1993). Subsurface drip irrigation: Realizing the full potential. JORGENSEN, G.S., NORUM, K.N. (1992). Subsurface drip irrigation. Theory, practices and application. Fresno: California Center of Irrigation Technology, 73-78.

Philip, J. R. (1966). Plant water relations: some physical aspects. Annual Review of Plant Physiology, 17(1), 245–268.

Phogat, V., J. Simunek, M. A. Skewes, J. W. Cox, and M. G. McCarthy. 2016. Improving the estimation of evaporation by the FAO-56 dual crop coefficient approach under subsurface drip irrigation. Agricultural Water Management, 178 (Dec): 189–200.

Poblete-Echeverría, C. A., Ortega-Farias, S. O. (2013). Evaluation of single and dual crop coefficients over a drip-irrigated Merlot vineyard (Vitis vinifera L.) using combined measurements of sap flow sensors and an eddy covariance system. Australian Journal of Grape and Wine Research, 19 (2), 249–260.

Porras, I., Brotons, J.M., Conesa, A., Manera, F.J. (2014). Influence of temperature and net radiation on the natural degreening process of grapefruit (Citrus paradisi Macf.) cultivars Rio Red and Star Ruby. Scientia Horticulturae, 173, 45-53

Prgomet, I., Pascual-Seva, N., Morais, M. C., Aires, A., Barreale, D., Castro-Ribeiro, A., Silva, A. P., Barros, A. I.R.N.A., Gonçalves, B. (2020). Physiological and biochemical performance of almond trees under deficit irrigation. Scientia Horticulturae, 261, 108990.

Provenzano, G. (2005). Discussion of "Analytical Equation for Variation of Discharge in Drip Irrigation Laterals" by V. Ravikumar, C. R. Ranganathan, and S. Santhana Bosu. Journal of irrigation and drainage engineering, 131, 300-302.

Provenzano, G. (2007). Using HYDRUS-2D simulation model to evaluate wetted soil volume in subsurface drip irrigation systems. Journal of Irrigation and Drainage Engineering, 133 (4), 342-349.

Prueger, J. H., Hatfield, J. L., Parkin, T. B. 2005. Tower and aircraft Eddy covariance measurements of water vapor, energy, and carbon dioxide fluxes during SMACEX. Journal of Hydrometeorology, 6 (6): 954–960.

Puértolas, J., Conesa, M.R., Ballester, C., Dodd, I.C. (2014). Local root abscisic acid (ABA) accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. Journal of Experimental Botany, 66, 2325–2334.

Puglisi, I., Nicolosi, E., Vanella, D., Lo Piero, A. R., Stagno, F., Saitta, D., Roccuzzo, G., Consoli, S., Baglieri, A. (2019). Physiological and Biochemical Responses of Orange Trees to Different Deficit Irrigation Regimes. Plants, 8, 423.

Radoglou, K.M., Jarvis, P.G. (1990). Effects of CO_2 enrichment on four poplar clones. II. Leaf surface properties. Annals of Botany, 65, 627–632.

Rafi, Z., et al. 2019. Partitioning evapotranspiration of a drip-irrigated wheat crop: Inter-comparing eddy covariance, sap flow, lysimeter and FAO-based methods. Agricultural and Forest Meteorology, 265: 310–326.

Rallo, G., Agnese, C., Blanda, F., Minacapilli, M., Provenzano, G. (2010). Agro-Hydrological models to schedule irrigation of Mediterranean tree crops. Italian Journal of Agrometeorology, 1, 11-21.

Rallo, G., Agnese, C., Minacapilli, M., & Provenzano, G. (2011). Comparison of SWAP and FAO agro-hydrological models to schedule irrigation of wine grapes. Journal of Irrigation and Drainage Engineering, 138(7), 581-591.

Rallo, G., Agnese, C., Minicapilli, M., Provenzano, G. (2012). Assessing AQUACROP water stress function to evaluate the transpiration reductions of olive mature tree." Italian Journal of Agrometeorology, 1, 21–28.

Rallo, G., González-Altozano, P., Manzano-Juárez, J., Provenzano, G. (2017). Using field measurements and FAO-56 model to assess the eco-physiological response of citrus orchards under regulated deficit irrigation. Agricultural Water Management, 180, 136-147.

Ramírez-Cuesta, J. M., Allen, R. G., Zarco-Tejadad, P. J., Kilic, A., Santos, C., Lorite, I. J. (2019). Impact of the spatial resolution on the energy balance components on anopen-canopy olive orchard. International Journal of Applied Earth Observation and Geoinformation, 74 (Feb): 88–102.

Reca, J., Roldan, J.., Alcaide, M., Lopez, R., and Camacho, E. (2001). Optimisation model for water allocation in deficit irrigation systems. I. Description of the model. Agricultural Water Management, 48, 103-116.

Remorini, D., Massai, R. (2003). Comparison of water status indicators for young peach trees. Irrigation Science, 22, 39-46.

Ren, R., Liu, G., Wen, M., Horton, R., Li, B., Si, B. (2017). The effects of probe misalignment on sap flux density measurements and in situ probe spacing correction methods. Agricultural and Forest Meteorology, 232 (Jan): 176–185.

Ribeiro, R.V., Machado, E. C., Santos, M. G., & Oliveira, R. F. (2009). Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. Environmental and Experimental Botany, 66(2), pp.203– 211.

Robles, J. M., Botía, P., Pérez-Pérez, J. G. (2016). Subsurface drip irrigation affects trunk diameter fluctuations in lemon trees, in comparison with surface drip irrigation. Agricultural Water Management, 165, 11-21.

Roccuzzo, G., Villalobos, F. J., Testi, L., Fereres, E. (2014). Effects of water deficits on whole tree water use efficiency of orange. Agricultural Water Management,140 (Jul): 61–68.

Roccuzzo, G., Stagno, F., Ferlito, F., Intrigliolo, F., Cirelli, G.L., and Consoli S. (2014). Deficit irrigation for enhancing 'Tarocco' orange fruit quality. XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1112. 179-186.

Roccuzzo, G., Stagno, F., Ferlito, F., Intrigliolo, F., Cirelli, G.L., Consoli, S. (2014b). Deficit irrigation for enhancing ‘Tarocco’ orange fruit quality. Acta Hortic. 179-186. XXIX

International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1112.

Roccuzzo, G., Villalobos, F. J., Testi, L., Fereres, E. (2014 a). Effects of water deficits on whole tree water use efficiency of orange. Agricultural Water Management, 140, 61–68.

Rodríguez-Gamir J, Intrigliolo DS, Primo-Millo E, Forner-Giner M (2010) Relationships between xylem anatomy, root hydraulic conductivity, leaf/root ratio and transpiration in citrus trees on different rootstocks. Physiologia Plantarum, 139:159–169.

Romero, P., Lafuente, M.T., Rodrigo, M.J. (2012). The Citrus ABA signalosome: Identification and transcriptional regulation during sweet orange fruit ripening and leaf dehydration. Journal of Experimental Botany.63, 695–709.

Romero, P., Navarro, J. M., Pérez, P. J. G., García-Sánchez, F., Gómez-Gómez, A., Porras, I., Martinez, V., Botia, P. (2006a). Deficit irrigation and rootstock: their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin. Tree Physiology, 26, 1537–1548.

Romero, P., Navarro, J.M., Pérez-Pérez, J.G., García-Sánchez, F., Gómez-Gómez, A., Porras, I., Martínez, V., Botìa, P. (2006b). Effects of deficit irrigation on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin on two rootstocks. Tree Physiology, 26, 1537-1548.

Romero, P., Navarro, J.M., García, F., and Botía, P. (2004). Effects of regulated deficit irrigation (RDI) during the preharvest period on gas exchange, leaf development and crop yield of mature almond trees. Tree Physiology. 24:303–312.

Romero-Conde, A., Kusakabe, A., Melgar, J.C. (2014). Physiological responses of citrus to partial rootzone drying irrigation strategies. Scientia Horticulturae,169, 234–238.

Roychoudhury, A., Paul, S., Basu, S. (2013). Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. Plant Cell Reports, 32 985–1006.

Rosa, R. D., Ramos, T. B., Pereira, L. S. (2016). The dual Kc approach to assess maize and sweet sorghum transpiration and soil evaporation under saline conditions: Application of the SIMDualKc model. Agricultural Water Management, 177 (Nov): 77–94.

Ruiz-Sanchez, M.C., Domingo, R., Castel, J.R. (2010). Review. Deficit irrigation in fruit trees and vines in Spain. Spanish Journal of Agricultural Research, 8, S5-S20.

Sabatti, M., Scarascia Mugnozza, G., Valentini, R. (1992). Sap flux measurement in trees and their ecological and sylviculture applications. Monti e Boschi (Italy).

Saeed, H., Grove, I.G., Kettlewell, P.S., Hall, N.W. (2008). Potential of partial root zone drying as an alternative irrigation technique for potatoes (Solanum tuberosum). Annals of Applied Biology, 152, 71–80.

Saitta, D., Vanella, D., Ramírez-Cuesta, J.M., Longo-Minnolo, G., Ferlito, F., Consoli, S. (2020). Comparison of Orange Orchard Evapotranspiration by Eddy Covariance, Sap Flow, and FAO-56 Methods under Different Irrigation

Strategies. Journal of irrigation and drainage engineering, 146, 05020002.

Salon, J.L., Mendez, J.V., Chirivella, C., Castel, J.R. (2004). Response fo Vitis vinifera cv. ‘Bobal’ and ‘Tempranillo’ to deficit irrigation. Acta Horticulturae, 640, 91-98.

Sampaio, A.H.R., Coelho Filho, M.A., Coelho, E.F., Daniel, R., Machado, V.V., Carvalho, G.C., Santana, J.A.D.V. & Santana Junior, E.B. (2010). Partial root drying as strategy for water use optimization in a' Tahiti' lime orchard under the semi-arid of Bahia State. In VI International Symposium on Banana: XXVIII International Horticultural Congress on Science and Horticulture for People 928 (pp. 329-336).

Sánchez Blanco, M.J., Torrecillas, A., León, A., Del Amor, F. (1989). The effect of different irrigation treatments on yield and quality of Verna lemon. Plant Soil, 120, 299-302.

Santos, T.P., Lopes, C.M., Rodrigues, M.L., Souza, C.R., Silva, J.R., Maroco, J.P., Pereira, J.S., Chaves, M.M. (2005). Effects of partial root-zone drying irrigation on cluster microclimate and fruit composition of field-grown Castelão grapevines. Vitis, 44,117-125.

Satriani, A., Loperte, A., Soldovieri, F. (2015). Integrated geophysical techniques for sustainable management of water resource. A case study of local dry bean versus commercial common bean cultivars, Agricultural Water Management, 162, 57-66.

Schiermeier, Q. (2014). The parched planet: water on tap. Nature, 510:326– 328.
Schmid, H. P. (1994). Source areas for scalars and scalar fluxes. Boundary Layer Meteorol. 67 (3), 293–318.

Schmid, H. P., and Bünzli, D. (1995). The influence of the surface texture on the effective roughness length. Quarterly Journal of the Royal Meteorological Society, 121, 1–21.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., & Hammel, H. T. (1965). Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science, 148(3668), 339-346.

Schwacke, R., Grallath, S., Breitkreuz, K.E., Stransky, H., Frommer, W.B., Rentsch, D. (1999). LeProT1, a transporter for proline, glycine betaine and γ-amino butyric acid in tomato pollen. Plant Cell, 11, 377–391.

Selahvarzi, Y., Zamani, Z., Fatahi, R., Talaei, A.R. (2017). Effect of deficit irrigation on flowering and fruit properties of pomegranate (*Punica granatum* cv. Shahvar). Agricultural Water Management, 192, 189-197.

Shalhevet, J., Levy Y. (1990). Citrus trees. In: Stewart BA, Nielsen DR, eds. Irrigation of agricultural crops. Madison, WI: American Society for Agronomy, 951–986.

Sharma, B., Molden, D., Cook, S. (2015). Water use efficiency in agriculture: measurement, current situation and trends. Managing Water Fertilizer Sustainable agricultural intensification, 39.

Si, J. H., Feng, Q., Zhang, X. Y., Chang, Z. Q., Su, Y. H., H. Xi, Y. (2007). Sap flow of Populus euphratica in a desert riparian forest in an extreme arid region during the growing season. Journal of Integrative Plant Biology, 49 (4): 425–436.

Siddique, KHM., Bramley, H. (2014). Water deficits: development. Encyclopedia of Natural Resources, 1–4.

Silveira, L. K., Pavão, G.C., dos Santos Dias, C. T., Quaggio, J. A., de Matos Pires, R. C. (2020). Deficit irrigation effect on fruit yield, quality and water use efficiency: A long-term study on Pêra-IAC sweet orange. Agricultural Water Management, 231, 106019.

Singh, H.P., Kaushish, S.P., Kumar, A (2000). Micro-irrigation. Proceedings of the International Conference on Micro and Sprikler Irrigation Systems, 8-10.

Sitch, S., Huntingford, C., Gedney, N., Levy, P. E., Lomas, M., Piao, S. L., Woodward, F. I. (2008). Evaluation of the terrestrial carbon cycle, future plant geography and climatecarbon cycle feedbacks using _ve Dynamic Global Vegetation Models (DGVMs). Global Change Biology, 14(9), pp.2015–2039

Sivakumar, B. (2011). Water crisis: from conflict to cooperation-an overview. Hydrological Sciences Journal, 56:531–552.

Slama, I., Ghnaya, T., Messedi, D., Hessini, K., Labidi, N., Savoure, A., Abdelly, C. (2007). Effect of sodium chloride on the response of the halophyte species Sesuvium portulacastrum grown in mannitol-induced water stress. Journal of Plant Research, 120:291–299.

Smith, D.M., Allen, S.J. (1996). Measurement of sap flow in plant stems. Journal of Experimental Botany, 47, 1833-1844.

Snyder, R.L., Paw U, K.T., Duce, P., Spano, D., Ferreira, M.I., Do Paço, T.A., and Connell, J.H. (2000). Measuring tree and vine et with eddy covariance. Acta Horticulturae. 537, 53-60.

Soegaard, H., Boegh, E. (1995). Estimation of evapotranspiration from a millet crop in the Sahel combining sap flow, leaf area index and eddy correlation technique. Journal of Hydrology, 166 (3–4): 265–282.

Sperdouli, I., Moustakas, M. (2012). Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of Arabidopsis thaliana to drought stress. Journal of Plant Physiologyant Physiology, 169:577–585.

Spreer, W., Ongprasert, S., Hegele, M., Wünsche, J. W., Müller, J. (2009). Yield and fruit development in mango (Mangifera indica L. cv. Chok Anan) under different irrigation regimes. Agricultural Water Management, 96, 574–584.

Srayeddin, I. and Doussan, C., (2009). Estimation of the spatial variability of root water uptake of maize and sorghum at the field scale by electrical resistivity tomography, Plant Soil, 319, 185-207.

Stagno, F., Intrigliolo, F., Consoli, S., Continella, A., Roccuzzo, G. (2015). Response of orange trees to deficit irrigation strategies: Effects on plant nutrition, yield, and fruit quality. Journal of Irrigation and Drainage Engineering, 141, 04015014.

Stagno, F., Roccuzzo, G., Allegra, M., Intrigliolo, F., Parisi, R., Cirelli, G., Consoli, S., Barbagallo, S. (2015). Deficit irrigation strategies: Preliminary assessment on a sicilian young orange orchard. Acta Horticulturae, 1065.

Steppe, K., Dzikiti, S., Lemeur, R., Milford, J.R. (2006). Stomatal oscillations in orange trees under natural climatic conditions. Annals of Botany, 97, 831–835.

Stevenson, F.J. (1994). *Humus Chemistry: Genesis, Composition, Reactions*; John Wiley & Sons: Hoboken, NJ, USA.

Stikic, R., Popovic, S., Srdic, M., Savic, D., Jovanovic, Z., Prokic, L., Zdravkovic, J. (2003). Partial root drying (PRD): a new technique for growing plants that saves water and improves the quality of fruit. Bulgarian Journal of Plant Physiology, 2003, 164–171.

Swanson, R. H., and D. W. A. Whitfield. 1981. “A numerical analysis of heat pulse velocity theory and practice.” Journal of Experimental Botany, 32 (1): 221–239.

Taiz, L., and Zeiger, E. (2002). Plant Physiology, 3rd Edn. Sunderland, MA: Sinauer.

Talluto, G., Farina, V., Volpe, G., Lo Bianco, R. (2008). Effects of partial rootzone drying and rootstock vigour on growth and fruit quality of ‘Pink Lady’ apple trees in Mediterranean environments. Australian Journal of Agricultural Research, 59, 785–794.

Taylor, N., Mahohoma, W., Vahrmeijer, J., Gush, M.B., Allen, R.G., Annandale, J.G. (2015). Crop coefficient approaches based on fixed estimates of leaf resistance are not appropriate for estimating water use of citrus. Irrigation Science, 33, 153–166.

Testi, L., Villalobos, F. J. (2009). New approach for measuring low sap velocities in trees. Agricultural and Forest Meteorology, 149 (3–4): 730–734.

Thompson, A. L., Martin, D. L., Norman, J. M., Tolk, J. A., Howell, T. A., Gilley, J. R., Schneider, A. D. (1997). Testing of a water loss distribution model for moving sprinkler systems. Transactions of the ASAE 40 (1): 81–88.

Tian, F., Yang, P., Hu, H., Dai, C. (2016). Partitioning of cotton field evapotranspiration under mulched drip irrigation based on a dual crop coefficient model. Water 8 (3): 72.

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., Storey, R. (2007a). Irrigation management and rootstock effects on navel orange [*Citrus sinensis* (L.) Osbeck] fruit quality. Agricultural Water Management, 91, 24-32.

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., Storey, R. (2007b). Irrigation management and rootstock effects on navel orange [*Citrus sinensis* (L.) Osbeck] fruit quality. Agricultural Water Management, 91, 24-32.

Trentacoste, E.R., Calderón, F.J., Puertas, C.M., Banco, A.P., Contreras-Zanessi, O., Galarza, W., Connor, D.J. (2018a). Vegetative structure and distribution of oil yield components and fruit characteristics within olive hedgerows (cv. Arbosana) mechanically pruned annually on alternating sides in San Juan, Argentina. Scientia Horticulturae, 240, 425–429

Turner, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status. Plant Soil, 58, 339–366.

Tyree, M.T., Sperry, J.S. (1988). Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?: answers from a model. Plant Physiology, 88, 574–580.

Van der Gulik, T. W. (1999). B. C. Trickle Irrigation Manual. B. C. Ministry Agric. and Food Res. Manage. Branch and Irrig. Industry Assoc. of British Columbia, Abbotsford, B. C., Canada, 321 pp.

Van Noordwijk, M., Lawson, G., Hairiah, K., & Wilson, J. (2015). Root distribution of trees and crops: competition and/or complementarity. Treeñcrop interactions, 2nd edition: agroforestry in a changing climate. CAB International, Wallingford, 221-257.

Vanella, D., and Cansoli, S. (2018). " Eddy Covariance fluxes versus satellite-based modelisation in a deficit irrigated orchard." Italian journal of agrometeorology, 23 (2), 41-52.

Vanella, D., Cassiani, G., Busato, L., Boaga, J., Barbagallo, S., Binley, A., Consoli, S. (2018). Use of small scale electrical resistivity tomography to identify soil-root interactions during deficit irrigation. Journal of Hydrology, 556, 310–324.

Vanella, D., Ramírez-Cuesta, J. M., Sacco, A., Longo-Minnolo, G., Cirelli, G. L., & Consoli, S. (2020). Electrical resistivity imaging for monitoring soil water motion patterns under different drip irrigation scenarios. Irrigation Science, 1-13.

Vanella, D., Ramírez-Cuesta, J.M., Intrigliolo, D.S., Consoli, S. (2019). Combining Electrical Resistivity Tomography and Satellite Images for Improving Evapotranspiration Estimates of Citrus Orchards. Remote Sensing. Remote Sensing, 11, 373.

Vaysse, P., Soing, P., Peyremorte, P. (1990). L'irrigation des arbres fruitiers, Centre technique interprofessionnel des fruits et legumes, Paris.

Vereecken, H., Binley, A., Cassiani, G., Revil, A., Titov, K. (2006). Applied hydrogeophysics. In: Revil A, Titov K (eds) NATO science series IV: earth and environmental sciences. Springer, Dordrecht, 383.

Villalobos FJ, Testi L, Moreno-Perez MF (2009) Evaporation and canopy conductance of citrus orchards. Agricultural Water Management, 96:565–573.

Vieweg, G. H., Ziegler, H. (1960). Thermoelektrische registrerium der geschwindigkeit des transpirationsstromes. Ber. Dtsch. Bot. Ges. 73: 221–226.

Wahbi, S., Wakrim, R., Aganchich, B., Tahi, H., Serraj, R. (2005). Effects of partial root zone drying (PRD) on adult olive tree (Olea europaea) in field conditions under arid climate. I. Physiological and agronomic responses. Agriculture, Ecosystems & Environment, 106, 289-301.

Webb, L.B., Whetton, P.H., Barlow, E.W.R. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. Australian Journal of Grape and Wine Research, 13 (3), 165-175.

Wilson, K., Goldstein, A., Falge, E., Aubinet, M., Baldocchi, D., Berbigier, P., Bernhofer, C., Ceulemans, R., Dolman, H., Field, C., Grelle, A., Ibrom, A., Law, B.E., Kowalski, A., Meyers, T., Moncrieff, J., Monson, R., Oechel, W., Tenhunen, J., Valentini, R., Verma, S. (2002). Energy balance closure at FLUXNET sites. Agricultural and Forest Meteorology, 113:223–243.

Xiloyannis, C. (1992). Relazioni acqua-terreno-pianta, in Frutticoltura generale, Reda, 344-370.

Xiong, J., Zhang, L., Fu, G., Yang, Y., Zhu, C., Tao, L. (2012). Drought- induced proline accumulation is uninvolved with increased nitric oxide, which alleviates drought stress by decreasing transpiration in rice. Journal of Plant Research, 125:155–164.

Xiong, L., Zhu, J. K. (2003). Regulation of abscisic Acid biosynthesis. Plant Physiology. 133, 29-36.

Xu, H.L., Qin, F.F., Xu, Q.C., Xu, R.Y., Wang, T.Y., Wang, R. (2011) Applications of xerophytophysiology in plant production: sub-irrigation improves tomato fruit yield and quality. Journal of Food, Agriculture and Environment, 9, 256–263.

Xu, Z., Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany, *59*, 3317–3325.

Yakushiji, H., Nonami, H., Fukuyama, T., Ono, S., Takagi, N., Hashimoto, Y. (1996). Sugar Accumulation Enhanced by Osmoregulation in Satsuma Mandarin Fruit. Journal of the American Society for Horticultural Science, 121, 466-472.

Zaher-Ara, T., Boroomand, N., Sadat-Hosseini, M. (2016). Physiological and morphological response to drought stress in seedlings of ten citrus. Trees - Structure and Function, 30: 985-993.

Zandalinas, S.I., Rivero, R.M., Martínez, V., Gómez-Cadenas, A., Arbona, V. (2016). Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. BMC Plant Biology, 16, 105.

Zegbe, J.A., Behboudian, M.H., Clothier, B.E. (2006). Responses of 'Petopride' processing tomato to partial rootzone drying at different phenological stages. Irrigation Science. 24, 203-210.

Zhang, J., Schurr, U., Davies, W. J. (1987). Control of stomatal behaviour by abscisic acid which apparently originates in the roots. Journal of Experimental Botany, 38 1174–1181.

Zhang, J., Davies, W. J. (1990). Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant, Cell & Environment, 13 277–285.

Zhang, H., Wang, D., Ayars, J.E., Phene, C.J. (2017). Biophysical response of young pomegranate trees to surface and sub-surface drip irrigation and deficit irrigation. Irrigation Science, 35, 425-435.

Zhang, J., Jia, W., Kang, S.Z. (2001). Partial rootzone irrigation: its physiological consequences and impact on water use efficiency. Acta Botanica. Boreali-Occidentalia Sinica 21, 191–197.

Zitouna-Chebbi, R., Mahjoub, I., Mekki, I., Ben Mechlia, N. (2017). Comparing evapotranspiration rates estimated from atmospheric flux, soil water balance and FAO56 method in a small orange orchard in Tunisia. Acta Horticulturae, 1150: 23–30.

La borsa di dottorato è stata cofinanziata con risorse del
Programma Operativo Nazionale Ricerca e Innovazione 2014-2020 (CCI 2014IT16M2OP005),
Fondo Sociale Europeo, Azione I.1 “Dottorati Innovativi con caratterizzazione Industriale”

UNIONE EUROPEA
Fondo Sociale Europeo

Ministero dell'Università
e della Ricerca

PON
RICERCA
E INNOVAZIONE
2014 - 2020