

Rapid stomatal response in lemon saves trees and their fruit yields under summer desiccation, but fails under recurring droughts

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ABSTRACT

Lemon (*Citrus limon*) is a fruit tree with major agricultural importance around the Mediterranean basin and is considered to be highly drought resistant. In this study, we tested the effect of two months summer-desiccation on physiological and yield parameters of mature lemon trees growing under Mediterranean climate during three consecutive years. We also examined the efficiency of current irrigation regime, which is based on reference evapo-transpiration. We measured leaf gas exchange and water potential (Ψ), monitored sap flow and soil moisture and followed flowering, fruit set and fruit size. Lemon trees showed an isohydric stomatal regulation, as stomata maintained leaf water potential >-2 MPa. Summer desiccation caused a gradual decrease in diurnal tree water use, starting immediately after cessation of irrigation, with leaf gas exchange practically halted at the end of the drought period. Tree function recovered following re-irrigation, and fruit yields were not reduced, but even mildly increased during the first year. In contrast, summer desiccation during two consecutive years caused long-term effects of tree activity decrease, significantly lower yield, main branch collapse and even tree mortality. Irrigation amounts matched closely tree water-use amounts; soil moisture was maintained around 26% (v/v); and irrigation responded dynamically to meteorological changes, indicating that current irrigation regime represents highly efficient water management. The lemon desiccation protocol relied on the physiological capacity of this species to avoid short-term drought effects through stomatal closure. Still, this protocol must be managed carefully, to reduce risk to trees and save yields.

1. Introduction

Lemon (*Citrus limon*) is a fruit tree with major agricultural importance around the Mediterranean basin and is considered to be highly drought resistant with wide hydraulic safety margins and a rapid stomatal response to environmental conditions (Pérez-Pérez et al., 2009). Stomata are known to be the most immediate regulators of leaf gas exchange in response to changes in environmental conditions. For example, different studies have found a decrease in leaf gas exchange in response to elevated vapor pressure deficit (VPD) in apple (Fanjul and Jones 1982; Francesconi et al., 1997), orange (Ribeiro et al., 2009), and olive (Proietti and Famiani 2002). Another set of studies have shown tight relationships between stomatal conductance (g_s) and leaf water

potential (Ψ) in olive (Perez-Martin et al., 2014), grapevine (Charrier et al., 2018) and avocado (Cardoso et al., 2020). Other factors that were found to affect stomatal conductance in fruit trees are light (Marini and Sowers 1990), drought (Charrier et al., 2018; Paudel et al., 2019), atmospheric CO₂ (Paudel et al., 2018), and temperature (Francesconi et al., 1997). *Citrus* species also show oscillations in leaf gas exchange during the day (Levy and Kaufmann 1976; Steppe et al., 2006; Dzikiti et al., 2007; Gonzalez-Dugo et al., 2014) due to non-synchronized changes in leaf water loss and leaf conductance, which induce fast cycles of stomatal closure and opening (Cowan 1972; Farquhar and Cowan 1974).

Leaf gas exchange is crucial for carbon uptake and sugar synthesis, which, in turn, is key for fruit production. To maintain optimal leaf gas

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exchange while maintaining minimal water consumption, efficient irrigation management is required. Regulated deficit irrigation (RDI), which refers to a reduction in irrigation amounts during specific phenological stages, as well as continuous deficit irrigation (CDI) were found to have a positive effect on canopy size, fruit load, fruit size and yield quality in various fruit tree species such as almond, pistachio, pear and olive (Marsal et al., 2002; Goldhamer and Beede 2004; Goldhamer et al., 2006; Tognetti et al., 2007; Iniesta et al., 2009; Azzeddine et al., 2019). For *Citrus* species, specifically mandarin and orange, RDI was found to improve water-use efficiency (WUE) with positive to no effect on fruit yield quantity and quality (García-Tejero et al., 2011; Panigrahi et al., 2014; Panigrahi and Srivastava 2016).

For lemon trees, one common RDI practice is the Verdelli practice (Barbera et al., 1985; Raveh 2008), where irrigation is stopped for two months during the summer (typically July–August). The result is autumnal flowering and off-season yield the following summer. Previous studies on *Citrus* species have reported that partial root-zone dehydration, i.e. alternating irrigation in a way that wets only half of the root-zone, resulted with increased water-use efficiency and no or little effect on fruit-yield quantity and quality or on physiological aspects of the trees (Hutton and Loveys 2011; Consoli et al., 2017). However, the complete cessation of irrigation during the summer, as required by the Verdelli method, might exert long-term effects on the trees, which is yet to be fully understood. During the rest of the year, orchards are typically irrigated according to reference evapo-transpiration rate, calculated from the Penman-Monteith formula (Monteith 1965; Allen et al., 1998). This method is based on evaporation from a reference surface (typically grass), which is then fitted to individual crops via specific crop coefficients. This coefficient is meant to represent the proportion of evapotranspiration of the specific crop relatively to the reference crop, and is used by farmers to convert climatic conditions into locally implemented irrigation practices. Despite the specification of the general model to individual crops, finding physiological factors that can be easily monitored and provide insight into the trees' physiological state, has the potential to further increase the efficiency of irrigation practices. Under hotter, drier climate, such improvement could be key for sustaining agricultural cultivation in certain areas of the globe.

The goals of this study were to (1) examine the effects of the Verdelli practice on physiological behavior of lemon trees (namely leaf gas exchange); and (2) to evaluate the optimization of irrigation management by coupling water supply to physiological parameters of the trees (rather than the current reliance on the Penman-Monteith model, which calculates water use based on environmental conditions). To do so, we withheld irrigation for ~two months in the dry summer during three consecutive years. We monitored leaf gas exchange (i.e. net carbon assimilation (A); transpiration (E); stomatal conductance (g_s); and intercellular CO_2 (C_i)), water potential (Ψ_l) and sap fluxes on the diurnal and seasonal scales, and quantified tree water-use. We also monitored fruit set and size (i.e. diameter) to identify consequences of the agricultural practice on fruit yield. We hypothesized that: (1) considering the conservative stomatal regulation (i.e. fast response to environmental changes) of this species, trees will show immediate response to desiccation by closing stomata and halting carbon assimilation; (2) as a result, summer-desiccated trees would have lower rates of fruit set and smaller fruits; and (3) current optimal irrigation regime, calculated based on the Penman-Monteith model, probably represents over-irrigation in terms of water saving, compared to actual trees' water use.

2. Materials and methods

2.1. Experimental design

An orchard experiment was conducted at the Weizmann Institute orchard during 2017–2019. The soil is deep Hamra, a well-drained, aerated soil rich in iron. The climate is thermo-Mediterranean with daily mean temperatures of 12.9 °C and 27.8 °C in January and August,

respectively. Annual precipitation is ~570 mm, confined to a wet season between October and April. The orchard is managed by the Weizmann Institute Gardening Dept. according to the same guidelines of commercial *Citrus* orchards, including irrigation and pruning. Fruits are regularly collected by the local community. The orchard has been used before for eco-physiological studies by another research group (Yang et al., 2018). Trees are ~20 years old and were planted in parallel rows, 4 m apart, with 4.5 m in-between rows. Twenty trees were selected for the experiment, 10 irrigated and 10 summer-desiccated (Fig. S1). To account for subsurface water movements, additional 28 trees (two trees were missing from one of the rows), acted as buffer trees, meaning that their irrigation was arrested for the periods when summer-drought was applied to their neighboring trees. A preliminary excavation around the stem of one lemon tree in the orchard showed that the majority of the roots were concentrated within a radius of 50–70 cm from the tree's trunk (Fig S1). Thus, we concluded that the different trees in the plot are fit to act as independent replicates for the purpose of statistical analyses. In order to examine the long-term effect of the Verdelli practice, the summer desiccation treatment was switched between the groups in the second year, and then repeated for the same trees on the third year. This way, we could examine both the long-term effect of summer desiccation and to test for potential damage it exerts when implemented for two consecutive years. The trees were irrigated according to the Ministry of Agriculture irrigation guidelines, i.e. with a diurnal amount determined by the actual atmospheric demand. During ~2 months in the summer (3 August – 20 September 2017, 9 July – 2 September 2018, and 4 July – 2 September 2019), the irrigation was stopped for half of the trees. In order to follow both diurnal and seasonal dynamics of tree water-use, measurements in 2017–2018 were conducted during monthly campaign days, when Ψ_l and leaf gas exchange were measured along the day, typically five times per day between 8:00 and 17:00. In addition, mid-day Ψ_l was measured weekly throughout 2017–2019. Sap flow was measured on five trees of each treatment during selected dates in 2018, and during most of the days in 2019. Flower development, fruit set, number of fruits and fruit size were measured between April and December during 2018–2019.

2.2. Environmental monitoring

Air temperature (T; °C), relative humidity (RH;%) and precipitation (mm) were taken from the nearest Israel Meteorological Service meteorological station, Beit Dagan station, ~10 km from the Weizmann orchard, and were used to calculate vapor pressure deficit (VPD; kPa) according to the following equation (Sadler and Evans 1989)

Additionally, SWC was monitored continuously using appropriate sensors (EC-5, Decagon Devices, WA, USA), at the rhizosphere (i.e. at ~15 cm depth, 0.5 m from the tree stem and 0.35 m from the drip irrigation valves), next to one tree at each of the irrigated and the summer-desiccated tree groups. The measurement depth was decided based on a previous study which found that changes in soil water content occur mainly at 30 cm depth below orange trees (Cohen and Cohen 1983) and the distance from the tree stem was decided based on the preliminary excavation shown in Fig. S1. A water retention curve was estimated based on parameters measured for the soil in the nearby experimental farm of the Faculty of Agriculture of The Hebrew University (1 km away from the lemon plot), and using the van Genuchten-Mualem model (van Genuchten and Nielsen 1987):

$$\frac{\theta - \theta_r}{\theta_s - \theta_r} = \frac{1}{1 + ((ah)^n)^m} \quad (1)$$

Where θ is the transient soil water content ($m^3 m^{-3}$); θ_r is the residual soil water content ($0.093 m^3 m^{-3}$); θ_s is the saturated water content ($0.359 m^3 m^{-3}$); α , n and m are constants determined based on the soil properties (equal to 0.0284 and 3.945, respectively for the soil in our research plot).

During leaf gas exchange measurements, photosynthetic photon flux

density (PPFD; $\mu\text{mol photons s}^{-1} \text{m}^{-2}$) was measured using the sensor of the gas exchange system (GFS-3000, Walz, Effeltrich, Germany).

2.3. Orchard irrigation

The orchard was irrigated from the end of the wet season (April-May) until the end of the dry season (November), using drip-irrigation system with 12 drippers of 1.6 L h^{-1} flow per tree. For half of the trees, irrigation was stopped for two consecutive months in the middle of this period, i.e. between July and September. Irrigation data was provided by the Weizmann Institute gardening department, for the entire orchard, at an area of 7.97 ha. These amounts were transformed to $\text{mm} (\text{dm}^3 \text{m}^{-2})$, and then multiplied by the area occupied by each tree (16 m^2 , based on plot area/number of trees) to calculate the irrigation amount per tree ($\text{dm}^3 \text{tree}^{-1}$). Since irrigation was applied once in 2–7 days, and typically every 3 days, we averaged the irrigation amount by the number of days between irrigation events, to calculate the time-averaged irrigation amount on a diurnal scale ($\text{dm}^3 \text{tree}^{-1} \text{day}^{-1}$).

2.4. Leaf gas exchange and water potential

Leaf gas exchange, i.e. assimilation (A ; $\mu\text{mol CO}_2 \text{s}^{-1} \text{m}^{-2}$), transpiration (E ; $\text{mmol H}_2\text{O s}^{-1} \text{m}^{-2}$), stomatal conductance (g_s ; $\text{mmol H}_2\text{O s}^{-1} \text{m}^{-2}$) and intercellular CO_2 (C_i ; ppm) were measured with a gas exchange system (GFS-3000, Walz, Effeltrich, Germany). Instantaneous water use efficiency (iWUE) was calculated as follows:

$$iWUE = \frac{A}{E} \quad (2)$$

PPFD and temperature were adjusted to ambient conditions and the CO_2 levels were set to $400 \mu\text{mol mole}^{-1}$. Measurements were taken on three trees per treatment, at five time points during the daytime (i.e. between 8:00 to 17:00), on eight campaign days along the years 2017–18, in order to reflect the diurnal and seasonal leaf gas exchange dynamics. These measurements represent the different fluxes on the leaf level with high precision, but they are limited in their scope in terms of (1) time – they are not continuous and represent a specific moment; and (2) robustness – they are taken on a single leaf per tree and therefore limited in their ability to represent the fluxes of the whole tree. To complement these measurements, a second approach was taken, relying on main stem sap flow measurements (see Section 2.5). Water potential was measured using a pressure chamber (PMS Instrument Company, OR, USA). For the measurements of Ψ_1 , leaves were taken from the tree at noon during weekly measurements and at five time points on seasonal campaign days. The leaves were placed in sealed plastic bags and kept on ice until brought to the lab and measured (maximum of 10 min period). The results of this protocol reflect roughly the most negative water potential experienced by the tree, rather than the stress experienced by the stem or even the branches. This distinction is important for the interpretation of these water potential measurements for irrigation management (Jones 2004).

2.5. Sap flow and canopy conductance

To complement the leaf-level measurements described in the previous section, sap flow measurements were used to calculate transpiration on the tree level. Sap flow was monitored in 2018 using lab-made thermal dissipation sensors (Granier and Loustau 1994; Paudel et al., 2013). In 2019, these sensors were replaced by commercial heat balance sensors (EMS, Brno, Czech Republic; Cermak et al., 1973, 1976). All sensors were installed at ten cm height in ten trees, five irrigated and five summer-desiccated. Sap flow rate was measured continuously and logged every ten minutes. With the thermal dissipation sensors, the temperature difference between probes was logged using a CR1000 data-logger (Campbell Scientific, UT, USA). Half-hour means and trees trunk circumference were used to calculate the sap flow rate per tree (L

$\text{h}^{-1} \text{tree}^{-1}$) as follows:

$$SF = 0.04284 \cdot \left(\frac{\Delta T_{max} - \Delta T_r}{\Delta T_r} \right)^{1.231} \cdot \frac{C^2}{4\pi} \quad (3)$$

Where SF is the half-hour mean of sap flow. ΔT_{max} is the maximal difference in temperature measured between the two probes during a 24 h period, used to set the baseline flow of each tree and regarded as “zero” or “negligible” flow. ΔT_r is the mean difference measured during the 30 min period. C the tree circumference in cm.

Canopy conductance (G_c , mm s^{-1}) was calculated from daytime (i.e. 11:00 - 15:00) half-hourly means of SF and VPD as done elsewhere (e.g. Oren et al., 1998; David et al., 2004). This computation assumes aerodynamic coupling of E for our outdoor dry climate, i.e. low values of the decoupling coefficient (McNaughton and Jarvis 1983), which has been shown to be the case for outdoors conditions and leaves the size of *Citrus* leaves (Wullschlegel et al., 2000; Monteith and Unsworth 2013, Table 13.2). In this case, the radiative component of evapo-transpiration is small, and therefore the shortened form of the inverted Penman-Monteith model (Granier et al., 1996) can be used to calculate G_c :

$$G_c = SF \cdot 10^{-1} \cdot \left(\frac{\lambda \cdot \gamma}{\rho \cdot C_p \cdot VPD} \right) \quad (4)$$

Where λ is the latent heat of vaporization of water (2.45 J kg^{-1}), γ is the psychrometric constant ($66 \text{ Pa } ^\circ\text{C}^{-1}$), ρ is the density of dry air (1.184 kg m^{-3}), and C_p is the specific heat of dry air at constant pressure ($1010 \text{ J kg}^{-1} \text{ } ^\circ\text{C}^{-1}$). For full derivation, see Paudel et al. (2015).

Canopy resistance (R_c ; s mm^{-1}) was calculated as the inverted value of G_c (mm s^{-1}):

$$R_c = \frac{1}{G_c} \quad (5)$$

R_c values were plotted against maximal daily VPD and the slope of the linear regression represented the whole tree hydraulic resistance (Cohen and Naor 2002; David et al., 2004; Bond et al., 2008; Paudel et al., 2015)

2.6. Flowering, fruit set, and fruit development

We monitored the number of flowers at peak flowering in mid-March 2018; the fruit set rate in late May 2018, following the late spring heatwaves; and the number of fruits at full size, in January 2019. The following season the same monitoring was done at April, August, October and Dec 2019 for number of flowers, fruit set rate and twice for fruits at full size, respectively. Two monitoring frames ($0.9 \times 0.9 \text{ m}$, i.e. 0.81 m^2 each) were constructed for each of the 20 study trees, on East and West sides of the crowns. In addition, fruit diameter was monitored on a weekly basis for five consecutive months, immediately following the summer desiccation treatment periods, i.e. during September 2017-January 2018; August-December 2018; and during May-November 2019. Fruit diameter was monitored using a digital caliper on 60 fruits growing on 3 irrigated and 3 summer-desiccated trees (10 fruits per tree).

2.7. Statistical analysis

In order to ensure that individual trees in the orchard represent independent replicates, a preliminary excavation was done around the trunk of one tree in the orchard (that was not used for physiological measurements). The excavation revealed that the majority of the root system was located within 50–70 cm radius of the base of the trunk (Fig. S1). In the past, it was shown that changes in soil water content occur mainly at 30 cm depth below orange trees (Cohen and Cohen 1983). For these reasons, we believe that the assumption of independent measurements, necessary for ANOVA, is met in this case. Statistical

analysis was carried out using the ezANOVA function in R software (Lawrence 2016, R Core 2018). The values of leaf gas exchange of the different trees from each treatment ($n = 3$) were averaged per time, date and treatment. The averaged values at mid-morning (i.e. $\sim 10:00$) from all seasonal campaigns were pooled and subjected to repeated measures ANOVA, where the different dates represented within-subject variance and the two treatments (i.e. summer irrigation and summer desiccation) represented between-subject variance. The effect of summer desiccation on physiological function of the trees was tested separately for the two campaign days that were carried out during the 2017 and 2018 droughts. Mid-morning values of each parameter (i.e. A, E, C_i , g_s and iWUE) were subjected to t -test, after ensuring normal distribution and homogeneity of the variances. To analyze the seasonal trend in Ψ_l , all measurements were taken into account, including weekly measurements and midday measurements on campaign day. The data were then subjected to repeated measures ANOVA using the ezANOVA function where the different dates represented within-subject variance and the two treatments represented between-subject variance. To test the effect of VPD on E, we used the linear model function in basic R software, for the mid-morning values pooled from each campaign day for each treatment and the respected VPD value from the meteorological data.

3. Results

3.1. Meteorological conditions during 2017–2019

The investigation period had typical conditions for the site, with temperatures ranging between 4 °C and 42 °C, and mostly around 12–22 °C on winter days and 25–33 °C on summer days (Fig. 1). VPD was mildest in mid-winter, ~ 0.5 kPa, and highest during spring and autumn heatwaves, up to 5.5 kPa. Rainfall was mostly confined to October–May, with diurnal amounts of up to 33 mm.

3.2. Soil water content and potential

Soil moisture at ~ 15 cm depth in the orchard mostly fluctuated around 20–25% (v/v), with transient peaks (pulses) of up to 38% in autumn 2017 (Fig. 1). The dry season irrigation, characterized by relatively fast fluctuations, maintained a moisture level of 23–32%, and typically around 26%. Cessation of irrigation during two summer months reduced soil moisture to 11–13%. The soil water potential, estimated from the calculated retention curve (Fig. S2), decreased to about -0.007 MPa during these periods. These values of water potential represent soil hydraulic conductivity of practically zero, as was shown by Assouline (2001) for the same type of soil. *Re*-irrigation increased soil water content levels back to that of irrigated trees rather quickly, in 2018 and 2019, or more gradually, in 2017. However, summer desiccation had a long-term effect on soil moisture, whereby moisture was

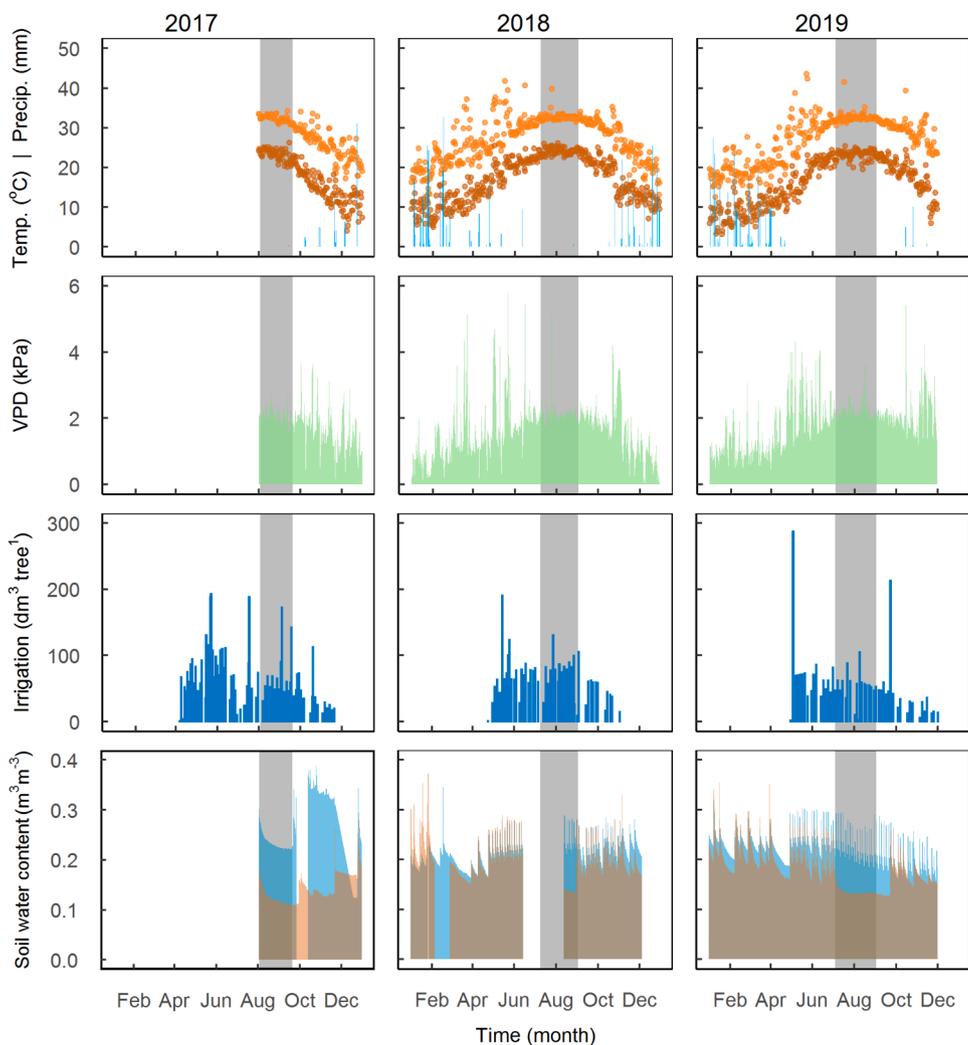


Fig. 1. Environmental conditions during 2017, 2018 and 2019 (left, middle and right columns, respectively) in the lemon orchard. First row: diurnal maximum and minimum temperatures (light and dark orange dots, respectively) and rainfall (blue bars); Second row: VPD, vapor pressure deficit; Third row: irrigation; Fourth row: soil water content at ~ 15 cm depth, 0.5 m from the tree stem, under irrigated trees (blue/light) and summer-desiccated trees (orange/dark). Gray shadings represent the desiccation period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

typically ~2% lower under the summer-desiccated tree in the following autumn. This difference was also shown in the soil water potential dynamics. Under the summer-desiccated tree, soil water potential tended to drop lower between irrigation events, by up to 0.002 MPa difference during June 2019 (Fig. S2).

3.3. Irrigation regime

Irrigation events translated into 50–200 dm³ tree⁻¹ once in 3–4 days and up to 300 dm³ tree⁻¹ on a few occasions (Fig. 1). Note that these values are later divided by the interval length (number of days in-between irrigation events) to produce the time-averaged diurnal irrigation amounts (Fig. 1). Overall, the 2017, 2018, and 2019 irrigation seasons summed into 732, 629, and 593 mm of irrigation, respectively. Notably, these amounts were moderately higher than the natural precipitation amount in the wet season (~570 mm), since they were designed to maintain a similar SWC at otherwise higher VPD (Fig. 1). In addition, the decrease in irrigation amounts during the three years reflects the dynamic irrigation responses to the changing meteorological conditions, i.e. milder conditions in the 2019 dry season compared to the 2018 dry season, and the increase in precipitation amount during the wet season along these years. Specifically, the 2018 wet season was unique in its high spring precipitation (Fig. 1), and the 2019 wet season was unique in its higher total amount. For these reasons, the 2018 and 2019 irrigation season started later than the 2017 irrigation (Fig. 1). Nevertheless, the 2019 irrigation continued longer into autumn, due to the lack of autumn rainfall and the unusually warm and dry days in autumn 2019 (Fig. 1).

3.4. Seasonal and diurnal dynamics of leaf gas exchange and water potential in irrigated and summer-desiccated lemon

Leaf mid-morning A was relatively stable throughout the year for irrigated trees, with values ranging between 5.5 and 7 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, except for January 2018, when values decreased to 2.0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (but then increased to 6.3 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 12:30; Figs. 2b, S3). In non-irrigated trees, midday A values fluctuated throughout the year, with values that were significantly lower compared to irrigated trees during the drought periods (*p*-value < 0.001 and = 0.01 for the drought periods of 2017 and 2018, respectively; see Table 1 for complete results of the repeated measured ANOVA) and a gradual increase together with the rehydration period, peaking at July, almost a year

Table 1

The effect of summer-desiccation on physiological parameters. Results of repeated measures ANOVA and t-tests for the differences in physiological parameters between summer-desiccated and irrigated trees along the year and specifically during 2017 and 2018 drought periods.

Parameter	Difference between treatments along the year		2017 drought		2018 drought	
	<i>p</i> -value	F-value	<i>p</i> -value	t-value	<i>p</i> -value	t-value
Ψ_1	0.03	10.97				
A	0.404	0.87	<0.001	-16.33	0.01	-4.10
E	0.55	0.43	0.01	-9.08	0.03	-3.40
C_i	0.14	3.38	0.001	7.84	0.02	3.91
g_s	0.66	0.23	<0.001	-9.64	0.04	-2.97
iWUE	0.76	0.11	<0.001	-8.74	0.04	-4.75

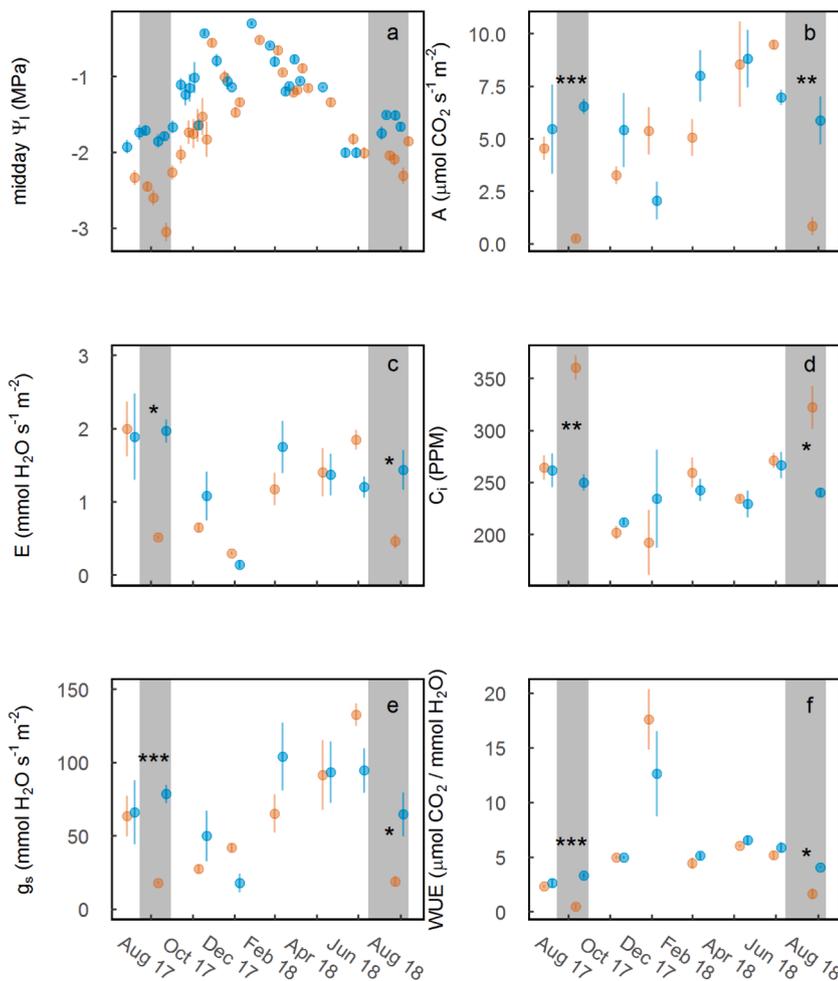


Fig. 2. Midday values of leaf water potential (Ψ_1), and mid-morning values of leaf gas exchange (A, net carbon assimilation; E, transpiration; C_i , intercellular CO_2 ; g_s , stomatal conductance; iWUE, instantaneous water use efficiency) during 2017–2018. Blue (light) and brown (dark) dots represent summer-irrigated and summer-desiccated trees, respectively ($n = 3$). Bars represent standard errors. Asterisks mark significant difference between summer-irrigated and summer-desiccated trees, according to the *p*-value of *t*-test ($\alpha = 0.05$; * = 0.01–0.05; ** = 0.001–0.01; *** = 0–0.001). Gray shadings indicate desiccation periods. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

after the drought has ended. Mid-morning E values were lower during the wet season in both irrigated and summer-desiccated trees, below $1 \text{ mmol m}^{-2} \text{ s}^{-1}$, and peaked to $\sim 2 \text{ mmol m}^{-2} \text{ s}^{-1}$ in the dry season (Fig. 2c). These seasonal dynamics matched the VPD dynamics (Fig. S4), although the trend was significant only for the irrigated trees, mainly due to the low E values of the summer-desiccated trees during the drought periods, when VPD was high. Again, significant differences were found between treatments only during the drought periods. Similar pattern was observed for g_s , with a decrease during the wet season and a significant difference between treatments only during drought periods (Fig. 2e). C_i of irrigated trees was quite stable throughout the year, ranging from 210 to 270 ppm, compared to that of summer-desiccated trees, that reached as high as 360 and 320 ppm during the 2017 and 2018 drought periods, respectively, representing the low levels of carbon assimilation during these times (Fig. 2d). In general, summer-desiccated trees reduced their g_s , and hence E, by about 70–80% during both drought periods, compared to irrigated trees. Interestingly, the effect on photosynthesis was higher, as it was reduced by $\sim 90\%$. Taken together, this means that summer desiccation reduced tree water-use efficiency, i.e. these trees lost more water per any amount of carbon gained. Indeed, instantaneous water use efficiency (iWUE) of irrigated trees was quite constant throughout the year, ranging from 2 to $6.5 \mu\text{mol CO}_2$ assimilation per $\text{mmol H}_2\text{O}$ loss, while during drought periods, summer-desiccated trees had a significantly decreased iWUE. In addition, both treatments showed elevated iWUE during January 2018, probably due to low E (Fig. 2f). Midday Ψ_1 of irrigated trees ranged from -0.3 MPa in Jan to -2.0 MPa in Jun and Jul, respectively. In summer-desiccated trees values ranged between -0.5 MPa in Jan (post summer-desiccation) to -3 MPa at the peak of drought treatment. However, in spite of these differences, midday Ψ_1 did not drop below -3 MPa (Figs. 2, S3, S5), suggesting a highly conservative hydraulic behavior.

On the seasonal scale, all leaf-level parameters (i.e. Ψ_1 , A, E, C_i , g_s and iWUE) showed a significant change (see Table 2 for the results of repeated measures ANOVA).

3.5. Sap flow dynamics, canopy conductance and resistance, and their sensitivity to environmental parameters

Diurnal curves of sap flow showed mostly a plateau shape, indicating maintenance of a constant water-use level along the day, around $2 \text{ L tree}^{-1} \text{ hr}^{-1}$ (Fig. 3a–c, Fig. 3d for irrigated trees and Fig. 3e for summer-desiccated trees). In late November of both 2018 and 2019, the shorter day meant a narrower activity window. At the end of the summer desiccation treatment, treated trees reduced their daily maximal water-use to $\sim 1 \text{ L tree}^{-1} \text{ hr}^{-1}$ (Fig. 3b–e). This difference was significant, yet not as large as the difference found in leaf transpiration (Figs. 2, S3). Considering the requirement for a closed water balance, it is possible that the instantaneous values of leaf gas exchange mildly overestimated the effect of the summer desiccation on tree water-use.

Long-term monitoring of sap flow during 2019 allowed to examine the relationship between sap flow and environmental conditions. The values seemed to stabilize at $\sim 4 \text{ L hr}^{-1} \text{ tree}^{-1}$ for irrigated trees, and at $\sim 2 \text{ L hr}^{-1} \text{ tree}^{-1}$ for the summer-desiccated trees following the drought treatment (Fig 4a,b–d). When coupled to the VPD through the calculated

Table 2

The effect of sampling date on physiological parameters. Differences in physiological parameters between different campaign days, as resulted from repeated measure ANOVA.

Parameter	p-value	F-value
Ψ_1	<0.001	2.82
A	0.007	4.45
E	0.005	4.68
C_i	0.015	3.72
g_s	0.014	3.82
iWUE	0.198	1.63

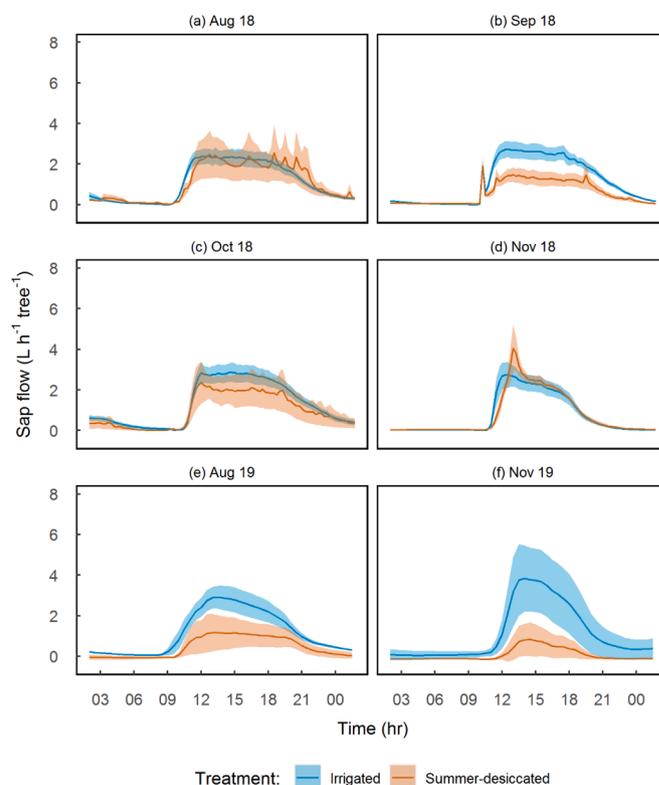


Fig. 3. Sap flow in irrigated (blue/light) and summer-desiccated (brown/dark) lemon trees using thermal dissipation (a–d) and heat balance (e–f) sensors during and after (a,b,e and c,d–f, respectively) summer desiccation. Values represent means \pm SE ($n = 4–6$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

G_c , a clear decrease in canopy conductance with increasing VPD was shown (Fig. 4c). Similarly, R_c increased linearly with VPD, with the slope of the curve rising from 1.1 in irrigated trees to 2.4 in summer-desiccated ones (Fig. 4e). The same trends were shown in the diurnal water use dynamics (Fig. 5). In the irrigated trees mean values were $17 \text{ L day}^{-1} \text{ tree}^{-1}$ during the summer (ranging from 8 to $23 \text{ L day}^{-1} \text{ tree}^{-1}$), and $10 \text{ L hr}^{-1} \text{ tree}^{-1}$ during late autumn (ranging from 5 to $12 \text{ L day}^{-1} \text{ tree}^{-1}$). Summer desiccated trees started with $12 \text{ L day}^{-1} \text{ tree}^{-1}$ before drought (ranging from 7 to $22 \text{ L day}^{-1} \text{ tree}^{-1}$) and decreased to a mean value of $5 \text{ L day}^{-1} \text{ tree}^{-1}$ during October–December (ranging from 3 to $8 \text{ L day}^{-1} \text{ tree}^{-1}$). Note that for summer-desiccated trees, 2019 was the second consecutive year of the treatment.

3.6. Tree water-use vs. water inputs in irrigation

To test the efficiency of the dynamic irrigation (which was performed in accordance with the Ministry of Agriculture guidelines) we calculated the ratio between the tree water-use (i.e. sap flow) and the time-averaged irrigation at the single-tree scale. Low ratios (below 1.0) might indicate a loss of irrigation water, e.g. to evaporation or runoff. This was the situation at the beginning of the dry season (Fig. 5c), with July ratios starting at 0.2 and increasing to 0.8. However, during most of the dry season, ratios fluctuated between 0.8 and 1.4, suggesting that the irrigation matched closely the actual tree water-use amounts. During autumn, tree water-use was actually higher than supplied by irrigation, reflecting the use of deeper soil water by the trees, which was later supported by rain events.

3.7. Flowering, fruit set, fruit development and tree mortality

By the spring following the summer drought treatment of 2018,

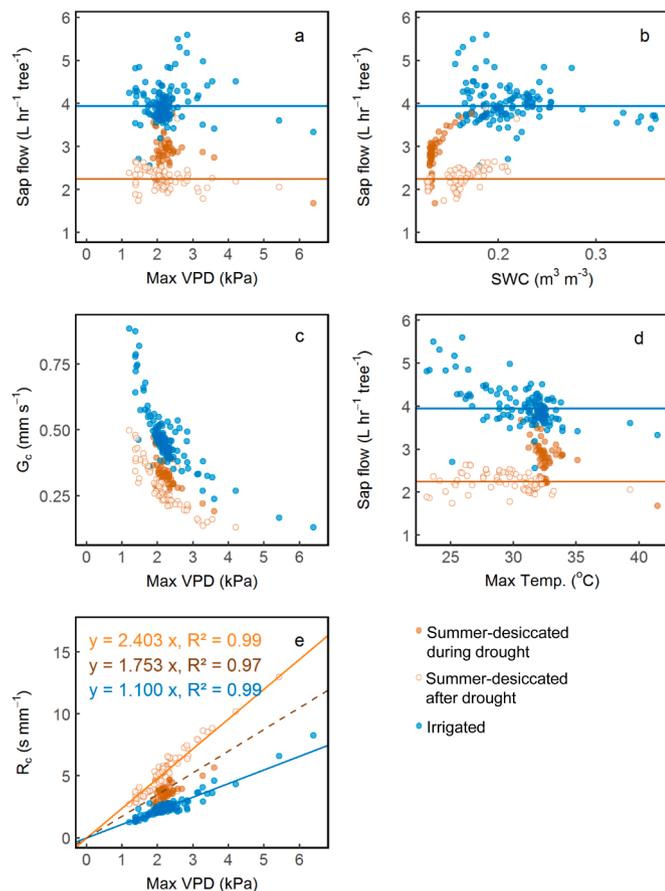


Fig. 4. Sap flow, canopy conductance (G_c) and canopy resistance (R_c) response to environmental conditions (i.e. daily maximum volumetric soil water content [SWC]; daily maximum temperature and daily maximum vapor pressure deficit [VPD]) measured during 2019 (a,b,d); each point represents the mean hourly sap flow per tree during peak daylight hours (i.e. 11:00–15:00). (c–e) each point represents a half hour mean of G_c and R_c during light-saturation hours. Blue/light circles represent irrigated trees; brown/dark circles represent desiccated trees under summer desiccation (full circles) or following the rehydration (empty circles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

summer-desiccated lemon trees had 30% less flowers than their irrigated neighbors (Fig. 6). The observed low fruit set rate of 12–15% is typical for *Citrus* (Sato 2015), however, trees of both groups produced a similar number of 20–21 fruits per m⁻² crown by January 2019 (Fig. 6a). Following the second consecutive year of summer desiccation, the treatment effects increased. Flower density, which remained at 180 flowers m⁻² at the irrigated trees, decreased by 45% in summer-desiccated trees, and this difference intensified at the fruit set and fruit stage. Finally, fruit density in December 2019 was 55% lower in summer-desiccated vs. irrigated trees (Fig. 6b). Fruit development, during summer and autumn, was rather similar across all trees in 2017, with 14% larger fruits in summer-desiccated than irrigated trees by the end of the year (Fig. 7). This pattern repeated when we switched the tree groups in 2018: fruit diameter on summer-desiccated trees was 12% higher than on irrigated trees. The difference between 2017 and 2018 was that the mild advantage in the summer-desiccated trees occurred earlier in the season: the groups diverged in November 2017, and then in September 2018. The second consecutive summer drought showed a contrasting pattern: fruit development was similar between the groups up until the beginning of the desiccation, in early July 2019, and then strongly diverged. Fruit development was completely arrested under drought, and resumed immediately after re-irrigation in September 2019. Simultaneously, fruits continued to develop on irrigated trees. By

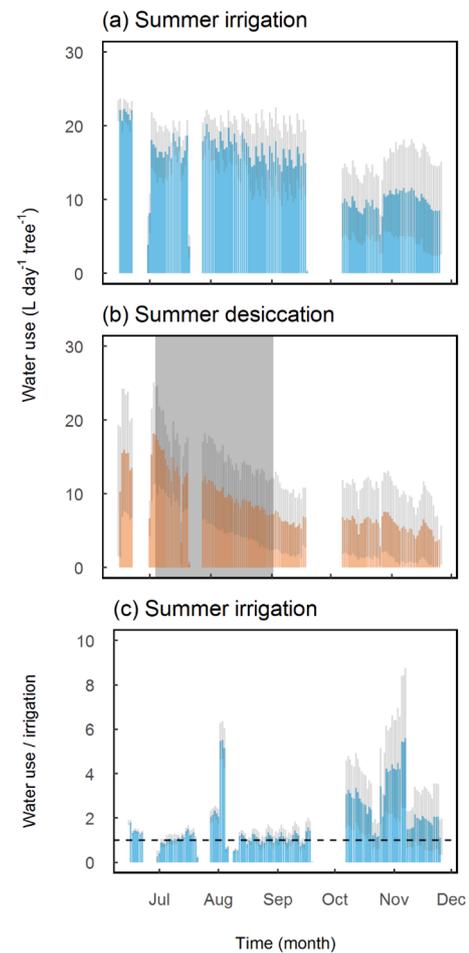


Fig. 5. Diurnal water-use amounts of irrigated trees (a) and summer-desiccated trees (b) in the lemon orchard in 2019, during the summer and after re-irrigation in early September. Gaps are due to breaks in the measurement system operation ($n = 4-6$). Gray shading in panel (b) indicates desiccation period. (c) The ratio between tree water-use and tree water supply by irrigation, for irrigated trees. The horizontal dashed line indicates the parity (ratio = 1.0). Gray bars represent standard error.

December 2019, fruit diameter was 27% higher in irrigated vs. summer-desiccated trees.

We have observed several incidents of tree mortality during and after the three years period of the experiment (Fig. S1). Among the measured trees, three trees have died following one summer-drought and additional three trees have died following two consecutive summer-droughts. We have also observed mortality among the buffer trees, i.e. trees that received the summer-desiccation treatments but were bordering with fully irrigated trees. In total, 11 trees out of the 48 used in our experiment died during or following the experimental period, of which 6 were among the 20 measured trees. Other trees in the orchard, that were not a part of our experimental plot, showed no signs of a biotic or abiotic stress throughout this period.

4. Discussion

Lemon trees have a high drought resistance, facilitating their survival in hotter and drier environments compared to other *Citrus* varieties (Cohen et al., 1997, Wagner et al. unpublished data). In this study we set out to (1) examine the effects of the Verdelli practice on physiological behavior of lemon trees (namely leaf gas exchange); and (2) evaluate the optimization of irrigation management by coupling water supply to physiological parameters of the trees (rather than the current reliance on

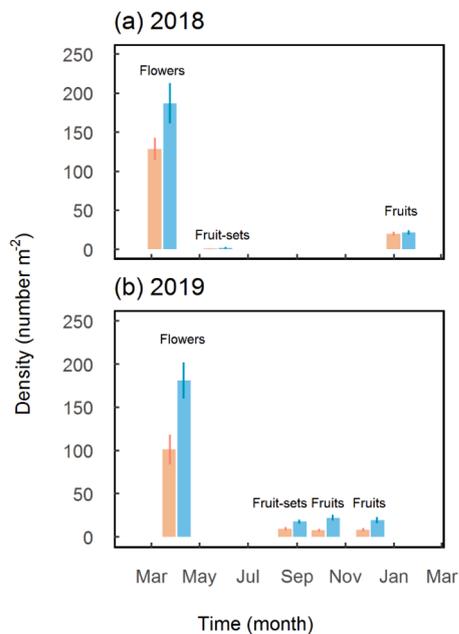


Fig. 6. The mean number of flowers, fruit sets, and fruits on irrigated (blue/light) and summer-desiccated (brown/dark) lemon trees. Bars represent standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the Penman-Montieth model, which calculates water use based on environmental conditions alone). We characterized the physiological patterns of lemon trees, in both irrigated and summer-desiccated scenarios and followed fruit set and development. Our results show that: (i) water use decreased gradually in summer desiccated trees, with values of leaf gas exchange reaching practically zero at the end of the drought period; (ii) Summer-desiccated trees had lower levels of fruit set and smaller fruits, but only following the second consecutive year of summer-desiccation treatment; (iii) The reference evapo-transpiration calculated from the Penman-Monteith formula proved as an appropriate method for setting irrigation regime for lemon trees under Mediterranean climate.

4.1. Stomatal and water use response to summer-desiccation

The stomatal response of mature lemon trees to dehydration started immediately after the cessation of irrigation at the middle of the dry summer (Fig. 5b). This response is shown by the gradual decrease in diurnal water use, and eventually resulted in leaf gas exchange values of practically zero after ~two months of dehydration, at the peak of the drought period (Fig. 2). A similar stomatal response was also shown in rain-fed olive trees by Marino et al. (2014). R_c was also found to increase significantly with increasing VPD (Fig. 4e). In addition, the elevated C_i in the summer-desiccated trees, together with the low A , is an indication for non-stomatal limitations to photosynthesis, i.e. biochemical constraints (Lawlor and Cornic 2002), as was found, for example, for *Ziziphus spina-christi* (Zait and Schwartz 2018). Observing the long-term effect of summer-desiccation, it seems that when applied for one year, the trees were able to recover full physiological function within 3–5 months, as observed during 2017–18 in leaf gas exchange (Fig. 2) and during 2018 in sap flow (Fig. 3d). However, when summer-desiccation was applied for two consecutive years, water use of summer-desiccated trees did not recover even after irrigation resumed, and stabilized on values about half of those of irrigated trees, even by December 2019, about three months after the end of drought (Figs. 3–5). On the other hand, under summer irrigation treatment, g_s remained constant across the seasons (Fig. 2), maintaining Ψ_l at or above -2.0

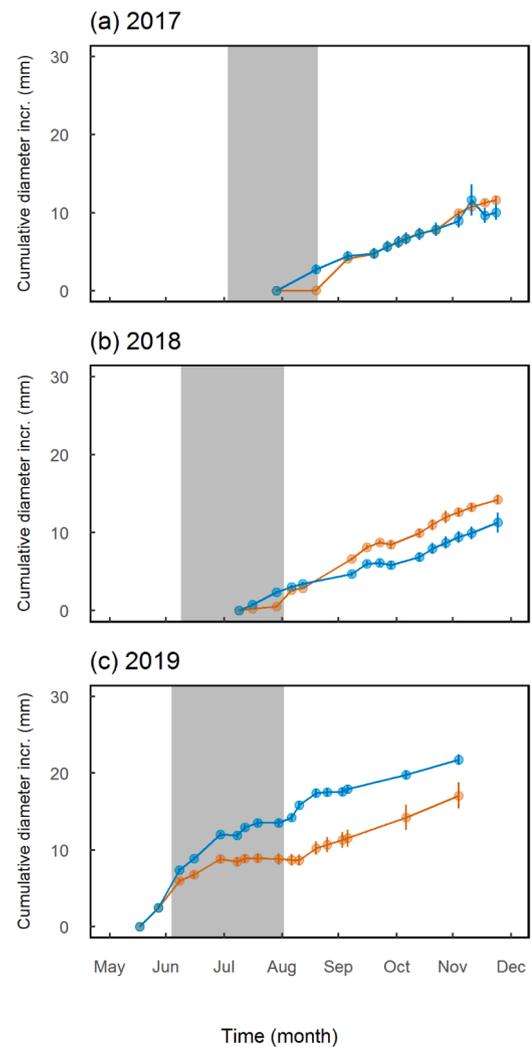


Fig. 7. Cumulative lemon fruit diameter increment in irrigated (blue/light) and summer-desiccated (brown/dark) trees in the lemon orchard during six months following the treatment. Values represent means \pm SE ($n = 30$). Gray shadings indicate desiccation periods. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MPa (Figs. 2, S3, S5). Additionally, the plateau shape of the diurnal sap flow curves implies that sap flow was maintained at a constant rate along the day in spite of the diurnal VPD fluctuations (Fig. 3), without any evidence of fast oscillations in leaf gas exchange previously reported for *Citrus* (Cowan 1972; Farquhar and Cowan 1974; Levy and Kaufmann 1976; Steppe et al., 2006; Dziki et al., 2007; Gonzalez-Dugo et al., 2014). This may indicate that stomatal oscillations are asynchronous and therefore are not apparent in integrative sap flow measurements. Alternatively, it is possible that the design of sap-flow measurements was not appropriate to monitor such fast changes in the leaves (i.e. in terms of the sampling interval or the location of the probes at the base of the trunk). In terms of canopy conductance, G_c decreased as VPD increased, as previously shown for this orchard (Yang et al., 2018). This relationship stabilizes sap flow, as indicated by the mid-day plateau (Fig. 3a–c,e). The shape of the G_c (VPD) curve was similar between the treatments, and conductance was higher for irrigated trees, and lower following desiccation (Fig. 4). This response was in line with that measured in deciduous orchards of apple and nectarine (Cohen and Naor 2002; Paudel et al., 2015, respectively) and in several forest tree species (Bond et al., 2008). Accounting for the performance of trees subjected to the two treatments, we suggest that lemon trees have a highly regulated stomatal functioning, characteristic of an isohydric behavior (Klein

2014). The constant g_s maintained by irrigated trees points to a tight relationship between stomata and VPD, which has already been shown for various fruit species (e.g. Cohen and Cohen 1983, Proietti and Famiani 2002, Ribeiro et al., 2009, Horna et al., 2011, Martin-StPaul et al., 2017). The significant decrease in g_s concurrently with the decrease in G_c implies that a large portion of the increase in R_c stems from stomatal resistance. However, further measurements are required to determine the proportion of the different components of R_c with and without summer-desiccation.

When examining leaf gas exchange compared to diurnal sap flow curves, a clear discrepancy emerges in the timing of peak activity. For example, during November 2017 leaf gas exchange of irrigated trees started to increase at 10:30 (or earlier), while water flow at the base of the stems during November of 2018 and 2019 started only at 11:00 (Figs. S3, 3d and 3f, respectively). Assuming these measurements are comparable (an assumption which is supported by the fact that diurnal sap-flow was similar between 2018 and 2019), there is a time lag of about 30 min between transpiration and sap flow. This may indicate that during this time, transpiration withdrawals on internal water, which is then refilled during the afternoon hours, when leaf gas exchange shuts down (at ~16:00) while sap flow continues (until about 19:00). Such a process has been shown for both angiosperm (Meinzer et al., 2004; Marino et al., 2014) and gymnosperm (Cermak et al., 2007; Klein et al., 2016) species. The fact that during November 2019 irrigation amounts did not meet the trees' water use (Fig. 5c), further strengthen the possibility that the trees had, to a certain extent, rely on stored water. Nevertheless, the fact that leaf gas exchange and sap flow were not measured at the same year requires that these conclusion will be further examined.

4.2. Cumulative effect of summer-desiccation on yield

The first year it was applied, the summer desiccation practice had little effect on final fruit number (Fig. 6), and a mild positive effect on fruit size (Fig. 7). However, imposing a second consecutive year of summer desiccation on trees resulted with undesired effects. Final fruit number decreased and the trees produced smaller fruits compared to the control trees, effectively impairing yield quantity. Previously it was found that reducing or withholding altogether irrigation during different stages of the reproductive cycle of *Citrus* species caused increased flower and fruit shedding, thus reducing yield (Kriedemann and Barrs 1981; Romero et al., 2006; Pérez-Pérez et al., 2008; García-Tejero et al., 2010). In contrast, other studies have found that light drought stress during fruit development had a positive effect on fruit quality in mandarin (Kaiharu et al., 2008; Iwasaki et al., 2011, 2012). For lemon, the effect of drought on fruit quality has not been studied yet. While we cannot determine the reason for the results shown in Fig. 7a,b, where applying summer-desiccation for one year resulted in larger fruits, we can hypothesize that the exact phenological stage of the trees plays a major role in the results of the summer-desiccation practice. For example, it was shown that RDI had a positive to no effect on fruit yield in various deciduous fruit trees (Goldhamer and Beede 2004; Goldhamer et al., 2006; Azzeddine et al., 2019) and a positive to no effect on fruit yield quantity and quality in mandarin (García-Tejero et al., 2011; Panigrahi et al., 2014; Panigrahi and Srivastava 2016). In addition to reduced flowers and yield, a number of trees lost main branches at the end of summer, in an abrupt, unexpected collapse (Fig. S6). Moreover, out of the 48 trees in our plot, 11 trees died during or following the experimental period (Fig. S1), suggesting further decrease in yield for years to come. It should be noted, however, that those affected trees had the largest crowns, and the loss of a main branch could be considered as self-pruning, i.e. a drought-induced adjustment of overgrown canopies. These trees have been growing with drip irrigation for two decades, limiting the development of their root systems, while facilitating high leaf area. This belowground/aboveground imbalance played a crucial role in the observed effects.). Finally, the drought legacy observed in sap

flow (Fig. 5) and in soil water content (Fig. 1) means that even physiological recovery would be delayed. Our results expose the limits for drought resistance in lemon represented by the harsh physiological, and consequently economic, effects of two consecutive years with summer-desiccation.

4.3. Efficiency of the summer irrigation regime

Is the reference evapo-transpiration method (Monteith 1965) a valid way to establish irrigation regime for lemon orchards? To answer this question, our study used irrigation amounts, tree water-use amounts, and SWC patterns. Integrating the three parameters together, we showed that the current irrigation regime is optimal to maintain stomatal activity in lemon during most of the summer. In turn, this activity translates into carbon uptake in photosynthesis (Fig. 2) and fruit production (Figs. 6,7), which is, otherwise, arrested. Three lines of evidence support the current irrigation regime: (1) irrigation amounts matched closely the actual tree water-use amounts in summer, i.e. without losses to evaporation or runoff (Fig. 5); (2) irrigation maintained a stable SWC around 26% throughout summer (Fig. 1); and (3) along the three years of the orchard experiment, irrigation has been highly dynamic, responding to meteorological conditions. Still, irrigation amounts seemed too high during Jul 2019 (Fig. 5), and there might be room for further adjustments. Previous studies have found no effect of RDI or CDI practices on either physiological activity nor fruit yield in deciduous fruit trees (Marsal et al., 2002; Azzeddine et al., 2019). Others have found that reduction in irrigation affects physiology but not fruit yield in deciduous and *Citrus* fruit trees (Goldhamer et al., 2006; García-Tejero et al., 2011; Panigrahi et al., 2014; Panigrahi and Srivastava 2016). It is possible that if summer-desiccation treatment in our study would be carried out in another manner (i.e. CDI or shorter and more precise RDI), the catastrophic outcomes of the second consecutive year of drought could be avoided.

Our results confirm the adequacy of the current irrigation regime for lemon orchards, in spite of our hypothesis of a water saving potential. Contrary to our expectations, we did not observe major water losses in the orchard, and water amounts were efficiently transferred into tree water-use. The Verdelli practice can temporarily improve fruit yield, while saving irrigation water. However, this practice can be risky, and must be avoided on consecutive years. It should be noted that, leaf water potential is not always a good indicator of drought stress, as was demonstrated by Jones and Sutherland (1991) and others, specifically midday Ψ_l , which is highly variable and responsive to external conditions. Here we showed fruit growth arrest and damage to branches in trees that maintained a water potential above -3 MPa. These observations mark the limits of drought resistance for this commercially important tree species.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Author contributions

YW managed the project under the supervision of TK and JG. Measurements of tree physiology and fruit diameter were performed and analyzed by YW, EP, PBO, and UR. Flowering and fruit set measurements were coordinated by ER and EN. Irrigation analysis was performed by SC. TK and YW wrote the paper, with contributions from all authors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2021.108487.

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