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Article

Response to Deficit Irrigation of 'Orogrande' Mandarin Grafted onto Different Citrus Rootstocks in Spain

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Abstract: Drought is increasingly becoming an abiotic stress factor that affects citrus yield in the Mediterranean Basin, and rootstocks may impact the plants' responses to it. This study compares the influences of Forner-Alcaide 5 (FA-5), an emerging well-established rootstock in Spain, and Carrizo citrange (CC), the common commercial rootstock of the Orogrande mandarin variety, on plants' responses to water reduction. The deficit irrigation (DI) condition was established by 50% irrigation and evapotranspiration (ETc). The canopy volume, yield, fruit size and fruit internal quality were evaluated. The yield reduction in the CC DI was mainly due to a smaller fruit size, but in FA-5, it was due to fewer fruits without an affected caliber. Regarding Ct, the fruits from the CC DI had lower juice contents and higher rind percentages, while the differences between the Ct and DI trees were smaller in the FA-5 rootstock. The most remarkable effect was the increase in total soluble sugars (TSS) for the DI treatment. To conclude, the FA-5 rootstock had the strongest influence on Orogrande mandarin under water stress. These results can be useful for addressing water stress problems in citrus.

Keywords: carrizo citrange; FA-5; quality; variety; water deficit; yield

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1. Introduction

Since water that is employed in irrigated agriculture accounts for more than 70% of all of the different uses worldwide, a minor reduction in irrigation water consumption could lead to a significant increase in its availability for remaining applications. The amount of water that is available for agriculture is shrinking due to land and water system degradation, competition with other economic sectors and the need to preserve aquatic ecosystem integrity. It is crucial to increase agricultural productivity through the sustainable intensification of groundwater extraction and, at the same time, to reduce the water and environmental footprints of agricultural production [1].

In addition, citrus trees are widely cultivated along the Mediterranean coast of Spain (298,000 ha in 2022; [2]), where irrigation is necessary for crops to be commercially viable due to the semi-arid climate [3], with a mean annual rainfall of 350–400 mm [4]. In this region, soil desertification is also rising [5], and climate change is increasing drought and flooding periods in the Mediterranean Basin's agro-systems [6]. Consequently, it is necessary to develop new irrigation scheduling strategies or techniques to optimize water use involving less water consumption, greater production and optimum fruit quality.

The most direct way to meet these requirements is to reduce water doses. Along these lines, new water management techniques and Regulated Deficit Irrigation (RDI) have been suggested as irrigation strategies to save water without lowering yields in a large number of crops, including citrus [3,7]. RDI is based on the principle that tree demands depend on both plant material and the phenological time during the crop cycle [8,9]. Citrus is considered a crop that is sensitive to water deficit [10], and considering the water demand in some phenological states, such as the flowering and fruit-set periods, is critical [11] because water deficit at these times normally compromises the yield by increasing the June fruit drop [8,12,13]. Furthermore, water restrictions in the last fruit growth and ripening phase might lower the yield by reducing the final fruit weight [9,13,14]. However, since the period after the June fruit drop is less sensitive to water restrictions [15], RDI applications should guarantee water supply in critical phenological states, but can restrict water supply when fruit growth is less sensitive to soil water deficit.

In addition, the crop response very much depends on the plant material, particularly the rootstock, which can also notably influence the variety of physiological responses to abiotic stress [11,16–18]. By way of example, plants undergoing soil water deficit often present physiological adaptive responses, such as decreased stomatal conductance, reduced transpiration and a rising leaf temperature [19]. When this adverse situation persists over time, citrus plants' growth, fruit production and juice quality decrease [20]. Thus, citrus breeding programs focus on developing new rootstock hybrids that are well adapted to water deficit, which saves water and makes crops become more ecological and sustainable systems. The hybrid Forner-Alcaide no. 5 (FA-5, 'Cleopatra' mandarin x *Poncirus trifoliate* (L.) Raf.) has been identified as a good alternative to citrange Carrizo (CC, *Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.) under salinity or iron chlorosis stresses and to water deficit under glasshouse conditions [16,21–23]. However, more research is needed to clarify the response of the whole scion/rootstock combination with water deficit and its influence on the production and quality of harvests under field conditions.

Given these considerations, the objectives of this work were to (1) evaluate the influence of the FA-5 rootstock, a recently well-established hybrid in Spain, on the Orogrande mandarin when subjected to water stress conditions and (2) compare it to CC, the most commonly used commercial rootstock, for the ultimate purpose of supplying the citrus breeding industry and rootstock nurseries with new plant material that is well adapted to this stress to allow for significant water savings without affecting fruit production or quality.

2. Materials and Methods

2.1. Plant Material and Field Experimental Design

This experiment was carried out in a field covering a surface area of 0.49 ha with adult trees of mandarin clementine cv. 'Orogrande' was grafted on two different rootstocks: citrange carrizo (CC) and Forner-Alcaide 5 (FA-5). Seeds came from the mother seed trees of the germplasm collection at the IVIA (Instituto Valenciano de Investigaciones Agrarias, Moncada, Spain). Buds were obtained from the germplasm collection at the IVIA. Rootstock seedlings were budded when they were 12 months old.

The field was located at the Agricultural Experimental Station of Carcaixent (39°6′45.7′′ N, 0°26′48.7′′ W, Valencia, Spain). Soil texture within the first 50 cm depth was classified as sandy (97.28% sand), with pH 8.69, $CaCO_3$ 5.34%, active calcium carbonate <1% and electric conductivity in the saturation extract at 25 °C of 0.06 mS cm^{-1}. The climate is Mediterranean, and meteorological data are shown in Table 1.

The employed irrigation system was localized drip irrigation. The plot was divided into four subplots: two were irrigated according to their water needs (100% crop ETc; control, Ct); the other two were irrigated at 50% of ETc (deficit irrigation, DI). Subplots were arranged alternately. Between two adjoining subplots, a row of border trees was left to withstand the influence of the two irrigation types. All of the subplots formed part of the same irrigation sector. Therefore, to apply deficit in the subplots with DI, the original dripper pipes of 4 L h^{-1} were replaced with others at a flow of 2 L h^{-1}. In this way, the subplots corresponding to DI received 50% of the water corresponding to Ct irrigation

(100% ETc) during the same irrigation period. The water pH was 7.8, electrical conductivity ranged from 2.0 to 3.5 mS cm^{-1} mS cm^{-1}, and B was 400–500 mg kg^{-1}.

Table 1. Meteorological data for the 4-year experiment (2018–2021).

	2018	2019	2020	2021
Mean Maximum Temperature (°C)	25.37	25.88	25.96	25.13
Mean Minimum Temperature (°C)	11.73	11.31	12.07	12.38
Relative Humidity (%)	70.39	68.6	71.8	73.7
Total Annual Evapotranspiration (ETo, mm)	1148.71	1176.15	1159.48	1081.25
Solar Radiation (MJ m^{-2} day^{-1})	16.67	17.19	16.56	15.26
Annual Rainfall (P, mm)	666.3	699.6	867.89	725.01

In each subplot, eight trees of each rootstock were selected for uniformity in size and lighting conditions. The total number of trees was 16 for each scion/rootstock treatment. Tree spacing was 6 m × 3 m. The plot was surrounded by border rows on all four sides (Figure 1).

Treatment	Column/Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Ct	2	B		**FA-5**		**CC**		FA-5		**CC**	**FA-5**			FA-5			CC		B
Ct	3	B		**FA-5**		**CC**		FA-5		**CC**	**FA-5**			FA-5			CC		B
	4	B																	B
DI	5	B			FA-5		CC				CC		**FA-5**	**CC**		**FA-5**	**CC**		B
DI	6	B		**CC**	**FA-5**	**CC**	**FA-5**		CC	FA-5	CC		FA-5			FA-5			B
	7	B																	B
Ct	8	B		FA-5	CC				CC	**FA-5**		FA-5		**CC**		CC	FA-5		B
Ct	9	B		**FA-5**	CC	**CC**				**FA-5**	**CC**	**FA-5**		**CC**			FA-5		B
	10	B																	B
DI	11	B			CC		FA-5	**FA-5**	**CC**			CC		**FA-5**	FA-5	**CC**			B
DI	12	B			CC		FA-5	**FA-5**	**CC**			CC		**FA-5**	FA-5	**CC**			B
	14	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Figure 1. Experimental plot with the distribution of subplots. The blue stripe corresponds to irrigation at 100% of the ETc (Ct). The orange stripe denotes deficit irrigation (DI, 50% ETc). The trees in bold represent those labeled for yield and fruit quality. CC: Carrizo citrange; FA-5: Forner-Alcaide no. 5; B: border tree.

Fertilization was supplied by an irrigation system and programmed following citrus standards [21] by considering the flow of the control trees and distributing it in weekly applications from March to September. The applied amounts were ammonium nitrate (33%) 2 kg $tree^{-1}$, mono-ammonium phosphate 0.5 kg $tree^{-1}$, KNO_3 0.7 kg $tree^{-1}$ and iron chelate 20 gr $tree^{-1}$. For the DI treatment, 50% of the fertilizer needs that could not be provided by the irrigation system were provided as a cover at the beginning of spring by distributing it around each tree. The other cultivation tasks, i.e., pruning, phytosanitary treatments, etc., were the usual ones for this variety and were identical in all of the subplots.

2.2. Irrigation Treatments

Irrigation doses were established based on the localized irrigation advisory program (PARloc) published on the website by the IVIA Irrigation Technology Service (http://riegos.ivia.es/) with the coefficients shown in Table 2 and calculated according to Equation (1).

$$\text{Irrigation doses} = (\text{ETc} - \text{Pef}) \times \text{Cins} = ((\text{ETo} \times \text{Kc}) - (\text{rain} \times \text{Fpe})) \times \text{Cins}. \quad (1)$$

where Kc is the crop coefficient that depends, in turn, on the % shaded area (PAs); Pef is the effective precipitation which depends on rain; Fpe is the effective precipitation factor and Cins is the installation constant that converts water needs from liters per square meter into irrigation hours. Irrigation frequency varied from 2 to 7 weekly irrigations, with the maximum frequency in summer months. During the October-February period, the water supplied by rain was sufficient, and no irrigation was carried out.

Table 2. Irrigation coefficients used to calculate irrigation doses, with Kc (crop coefficient), PAs (percentage of shaded area), Fpe (effective precipitation factor) and Cins (the installation constant).

	2018	2019	2020	2021
Kc	0.67	0.65	0.65	0.66
PAs	60%	70%	60%	65%
Fpe	0.75	0.80	0.75	0.80
Cins	0.75	0.60	0.75	0.60

The irrigation installation consisted of two dripper lines per row of trees, with the drippers separated by 1 m so that each tree was irrigated by six drippers. The change in dripper pipes from 4 to 2 L h^{-1} in the subplots with water deficit was made on 1 August 2018. So, the water restriction treatments began as of this date, and the duration was expected to cover a 4-year period (2018–2021).

To monitor and control the amount of water incorporated into the soil–plant system, four capacitance probes were installed (ECH2O EC-5 capacitance sensors connected to an Em50 data logger using the ECH2O Utility software 1.6 version, Decagon Devices, Inc., Pullman, WA, USA), with one for each irrigation pattern and dose, with volumetric humidity sensors at depths of 10, 30, 50 and 70 cm. Information on the moisture content and irrigation water was transmitted to a computer every 15 min. The sensors located in the first 50 cm of soil indicated the moisture content in the area of greatest root activity, while the sensor located at 70 cm depth indicated possible water losses due to percolation. The amount of water provided by irrigation in the control subplots (100% Etc.) had to be such that it maintained the moisture content of the first 50 cm of depth, between the field capacity values and 10–20% lower without reaching the increase in humidity at the 70 cm depth, because this would indicate that losses occurred due to percolation.

2.3. Tree Growth

At the end of each campaign, the diameters of the canopy (DT, transversal; DL, longitudinal to the planting line in m) were measured in two perpendicular directions, N-S and E-W, as was the total tree height (HT, in m) and the height to the lower branches (HF in m) of trees. The canopy volume (Vc, in m^3) was calculated using Turrell's formula (Equation (2) [16]):

$$\mathrm{Vc}\ (\mathrm{m}^3) = 0.524 \times (\mathrm{HT} - \mathrm{HF}) \times [(\mathrm{DT} + \mathrm{DL})/2]^2 \quad (2)$$

2.4. Yield Parameters

Some yield parameters, including fruit number (in fruit $tree^{-1}$), fruit fresh weight (in g) and yield (kg $tree^{-1}$), were determined at the end of the four campaigns (2018–2021) during the commercial fruit harvests. The mandarin samples were collected between November 25 and 30. The fruits from each tree were harvested, weighed and counted to calculate the yield per tree and the average fruit weight, which is obtained by dividing the total weight by the number of fruits per tree.

2.5. Fruit Size Determinations

To determine fruit quality, prior to harvesting, a sample of 12 fruits per tree was taken (3 fruits from each cardinal canopy position), which gave a total of 96 fruits per rootstock/irrigation combination. Fruits were mixed, and four subsamples (24 fruits each) were taken. One subsample (n = 24) was selected to measure physico-chemical characteristics using normal analysis procedures. Fruit fresh weight (FW, in g) was measured on a digital balance (Sartorius, Model BL-600, 0.01 g accuracy). Fruit diameter (D, in mm), fruit height (H, in mm) and peel thickness (in mm) were measured using an electronic digital slide gauge (Mitutoyo, Model CD-15 DC, 0.01 mm accuracy). The fruit shape index (D/H) was calculated.

For the distribution of diameters, four trees were randomly selected based on pattern and irrigation dose, and the diameters of 100 randomly chosen fruits around the tree were determined. Measurements were taken with a digital vernier caliper (0.01 mm accuracy).

2.6. Fruit Internal Quality

Three subsamples (24 fruit each) were used to determine the quality characteristics in juice. Fruits were squeezed together (3 replicates per each stock/irrigation combination, n = 3) using an electric squeezer. The juice, peel and rag percentages were measured. Total soluble sugars (TSS, in °Brix) were measured using a refractometer (Model N-1, Atago (0.2 °Brix)) and expressed as a percentage at 20 °C. The method used to analyze the Acidity Index (AI) was based on neutralization (NaOH 0.1 N) to pH 8.1. The Ripening Index (RI) was calculated as the TSS/AI ratio.

2.7. Statistical Analysis

Statistical analyses were performed using Statgraphic Centurion XVII (Statistical Graphics Corporation 2014) using the rootstocks/irrigation combination as the factor of analyses. A basic descriptive statistical analysis was followed by an analysis on variance test for mean comparisons. The Duncan test method was used to discriminate among means at a 95% confidence interval ($p < 0.05$). One correlation analysis of the parameters measured in 2021 was subjected to linear regression, and correlation coefficients (r) were determined.

3. Results

3.1. Canopy Volume

Vc (Figure 2) increased during the 4-year experiment in both the Ct rootstocks (8.9 m^3 and 7.5 m^3 in CC and FA-5, respectively), but without differences between them (61.6% and 65.8% increases, respectively). The Vc of the DI trees also increased, but to a lesser extent. In the DI trees, the increments in the crown volume between 2018 and 2021 in CC and FA-5 were 27.1% and 33.6%, respectively. This implies reductions in Vc in the CC and FA-5 of 35.5% and 32.1% compared to the Ct plants, respectively.

3.2. Yield Parameters

The number of fruits per tree was not influenced by the rootstock nor the DI treatment during the experiment (Table 3). Under the Ct conditions, the trees on FA-5 obtained higher fruit FW values than on CC in 2018 and 2019, and the mean value from the 4 years also rose (6.0% increase). DI lowered the fruit FW (around 14% reduction) in both rootstocks compared to the Ct plants during the experiment.

Yield (Table 3) was not affected by the rootstock under the Ct conditions, but differed when the DI treatment was applied. While DI reduced the yield in the CC plants at the end of the experiment, it lowered the yield in FA-5 in 2018 and 2021. DI significantly lowered the mean yield value in CC, but not in the FA-5-treated plants.

3.3. Fruit Quality

3.3.1. Fruit Physical Parameters

Both of the Ct rootstocks induced a similar fruit diameter (D, Figure 3A) during the experiment (61.2 ± 1.0 mm). DI reduced the fruit diameter parameter, but no significant differences were found between the DI plants. The mean reductions between the Ct and DI plants were 5.8%, 6.0% and 10.3% for the 2019, 2020 and 2021 harvests, respectively.

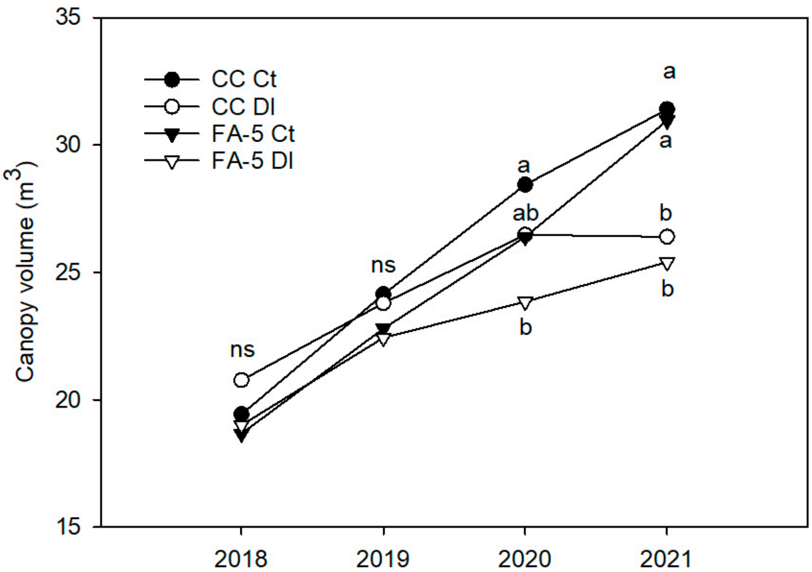

Figure 2. Canopy volumes (m^3) in the mandarin 'Orogrande' trees grafted onto rootstocks CC and FA-5 and grown under the control (Ct) conditions and with deficit irrigation (DI, 50% ETc) treatment, measured from 2018 to 2021. Data are shown as the mean values for $n = 8$ plants. For each year, the different letters between treatments indicate significant differences at $p < 0.05$ (LSD test); ns: not significant.

Table 3. Fruit $tree^{-1}$ number, fruit fresh weight (FW, g) and yield (kg $tree^{-1}$) in the mandarin 'Orogrande' trees grafted onto the CC and FA-5 rootstocks grown under the control (Ct) conditions and with the deficit irrigation (DI, 50% ETc) treatment determined from 2018 to 2021. The mean value of the 4 years was also calculated. Data are shown as the mean values for $n = 8$ plants. For each year, the different letters between treatments indicate significant differences at $p < 0.05$ (LSD test).

	Rootstock/Treatment	2018		2019		2020		2021		Mean	
Fruit $tree^{-1}$ number	CC Ct	1086	a	890		761	-	1269		1001	
	CC DI	969	ab	873		808	-	1468		1029	
	FA-5 Ct	933	ab	843		840	-	1335		988	
	FA-5 DI	709	b	828		894	-	1399		958	
Fruit FW (g)	CC Ct	90	b	94	b	107	a	95	a	99	b
	CC DI	82	c	87	b	95	b	72	b	85	d
	FA-5 Ct	100	a	103	a	110	a	101	a	105	a
	FA-5 DI	99	a	94	b	97	b	98	b	97	b
Yield (kg $tree^{-1}$)	CC Ct	98	a	84		81		120	a	95	a
	CC DI	80	ab	76		76		106	b	86	b
	FA-5 Ct	94	a	87		93		135	a	105	a
	FA-5 DI	75	b	82		90		111	b	91	ab

As a general trend, no statistical differences were observed in the fruit diameter/height (D/H) ratio (Figure 3B) or for the peel thicknesses between rootstocks or DI treatments. DI significantly reduced the peel thickness (Figure 3C) in the fruits collected in 2020, but only in the CC plants (7.1% decrease).

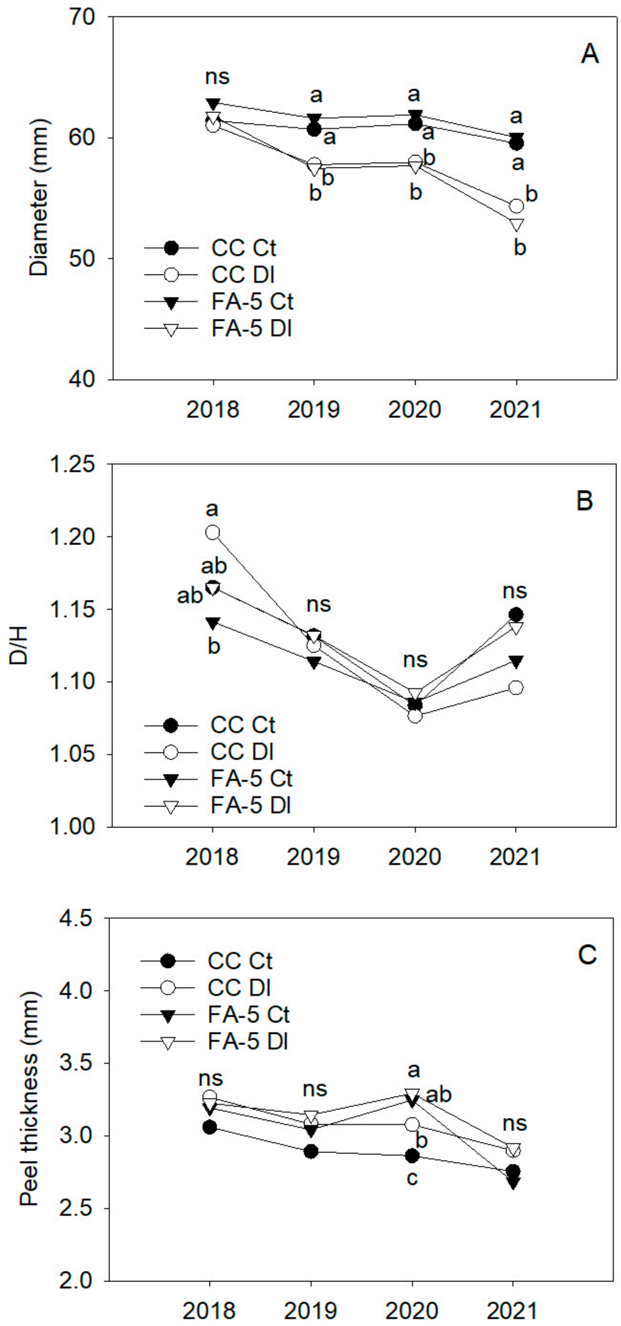

Figure 3. Fruit Physical Parameters. (**A**) Fruit diameter (D, mm), (**B**) diameter/height ratio (D/H) and (**C**) peel thickness (mm) in the mandarin 'Orogrande' trees grafted onto CC and FA-5 rootstocks and grown under the control (Ct) conditions and with deficit irrigation (DI, 50% ETc) treatment, determined from 2018 to 2021. Data are shown as the mean values for $n = 8$ plants. For each year, different letters indicate significant differences at $p < 0.05$ (LSD test); ns: not significant.

The fruit diameter distribution was similar for the CC and FA-5 plants under the Ct conditions (Figure 4). In 2019 and 2021, 50–55% of the Ct fruits had diameters of 54–62 mm (commercial categories II and III). In 2020, the fruits were slightly bigger, and 55% of their diameters ranged from 58 to 66 mm (commercial categories 1-X and II). In this year, 20.3% and 21.4% of the fruits from CC Ct and FA-5 Ct, respectively, fell into the highest commercial categories (>66 mm), which reduced by half or by 4-fold in 2019 and 2021 depending on the year and rootstock.

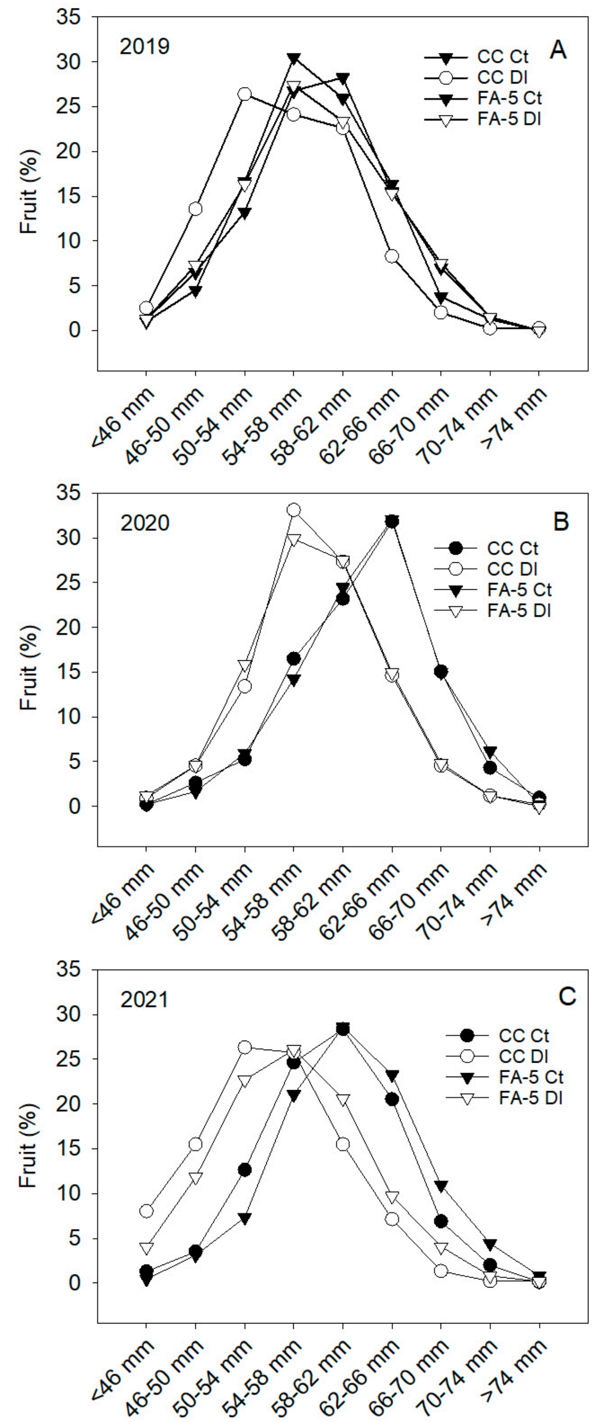

Figure 4. Distribution of fruit diameters (%) in the mandarin 'Orogrande' trees grafted onto CC and FA-5 rootstocks and grown under the control (Ct) conditions and with deficit irrigation (DI, 50% ETc) treatment determined in the fruits from the (**A**) 2019, (**B**) 2020 and (**C**) 2021 harvests. Data are shown as the mean values for n = 8 plants.

Under the DI conditions, the size distribution graph obtained during the 2019 campaign clearly showed a shift to the left, i.e., toward smaller sizes, for the fruits from the CC DI treatment because 39.9% of the fruits' diameters ranged from 46 to 54 mm (commercial categories IV and V). In general, the FA-5 DI plants were similar in size to the CC Ct and FA-5 Ct plants in 2019, and the FA-5 DI fruits in these categories did not exceed 23.6%. This tendency changed in 2020 and 2021, when the fruit size was affected by DI for both rootstocks. Between 67.6% and 73.9% of the DI fruits ranged from 50 mm to 62 mm in diameter (commercial categories II, III and IV), and a diameter of >62 mm was recorded only in 9–20% of the fruits depending on the year and rootstock, while this range was 29.6–53.4% in the Ct fruit.

The fresh peel percentage (Figure 5A) was similar in the fruits from the plants on both rootstocks grown under the Ct conditions. DI increased this parameter during three of the four harvests (2018, 2020 and 2021). The most marked rise occurred at the end of the experiment (2021), with around 26% for both DI treatments compared to the Ct plants. The peel percentage in dried biomass terms (Figure 5B) was more stable during the experiment, and differences between rootstocks were observed only for the last harvest. The control treatments had a similar dried peel value, which rose under the CI conditions by 19.5% and 22.6% in CC and FA-5, respectively.

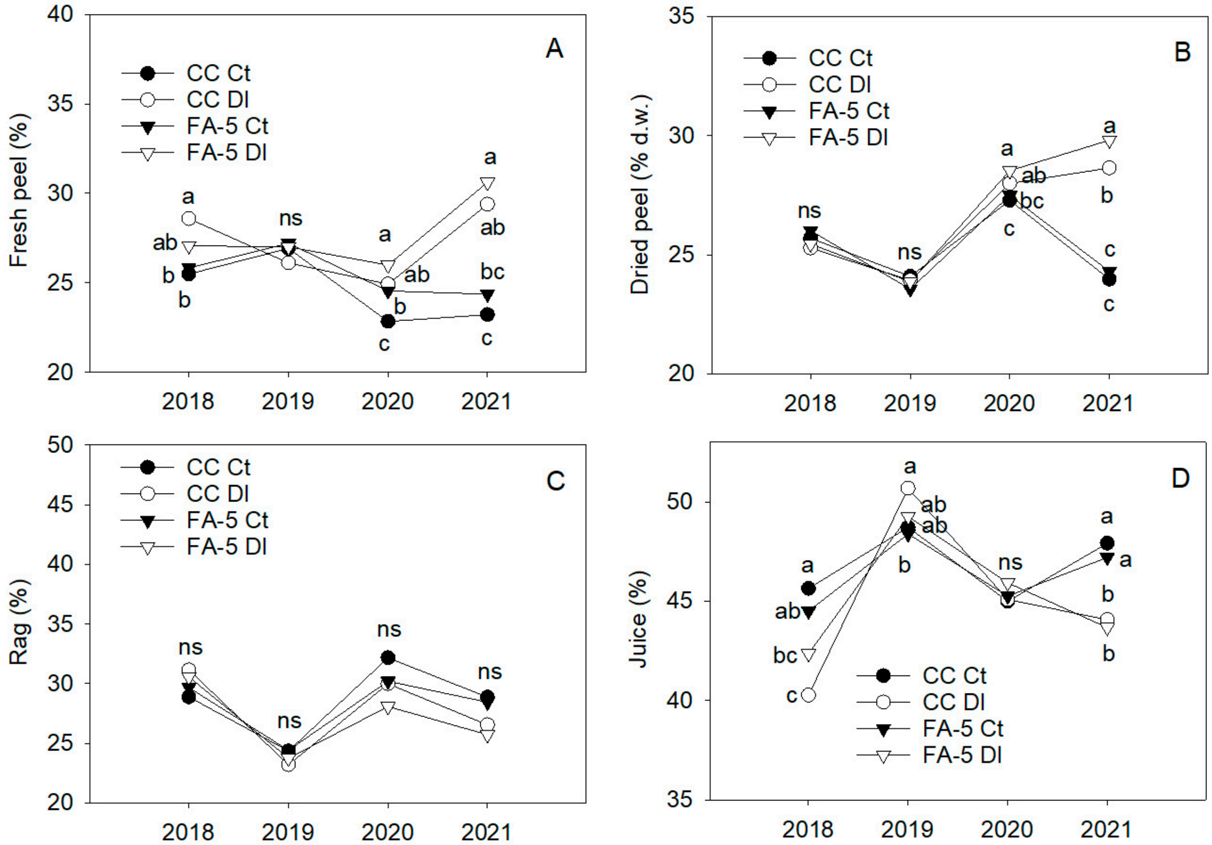

Figure 5. The fresh (**A**) and dried peel (**B**), rag (**C**) and juice (**D**) percentages (%) in the mandarin 'Orogrande' trees grafted onto CC and FA-5 rootstocks and grown under the control (Ct) conditions and with deficit irrigation (DI, 50% ETc) treatment determined from 2018 to 2021. Data are shown as the mean values for n = 8 plants. For each year, different letters indicate significant differences at $p < 0.05$ (LSD test); ns: not significant.

The rag percentage (Figure 5C) in the fruit was not influenced by the rootstock nor the DI treatment for any harvest. DI lowered the juice percentage (Figure 5D) in 2018 and 2021 by 8.3% and 7.7%, respectively, but without differences between rootstocks.

3.3.2. Juice Quality Parameters

The AI was similar in the plants grafted onto both Ct rootstocks (Figure 6A), and DI increased the AI during three of four harvests, but with no differences between the rootstocks at the end of the experiment. The mean increments in the AI parameter in the DI plants were 21.4% and 36.4% for 2020 and 2021, respectively, compared to the mean Ct values.

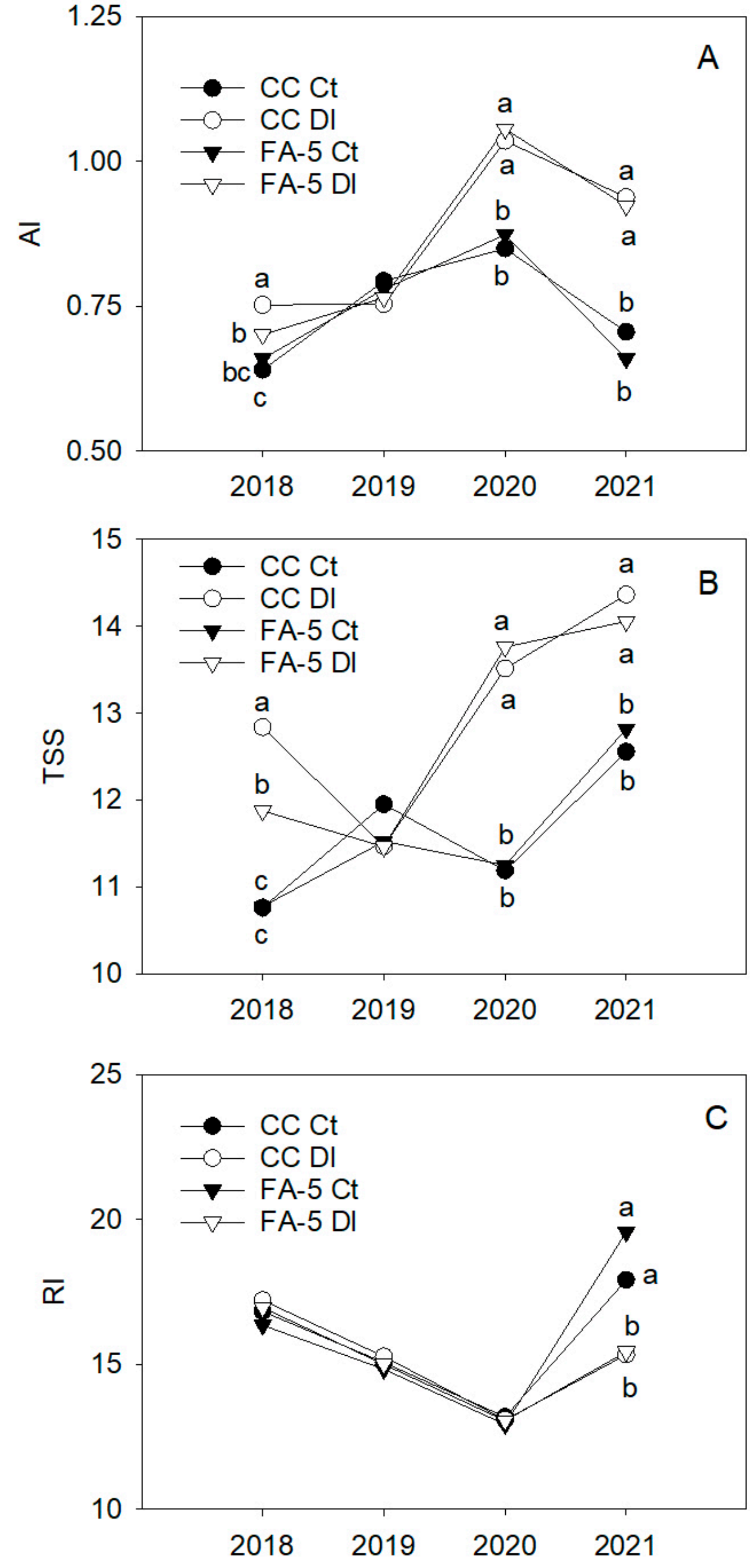

Figure 6. Acidity Index (AI) (**A**), total soluble sugars (TSS) (**B**) and Ripening Index (RI) (**C**) in the mandarin 'Orogrande' trees grafted onto CC and FA-5 rootstocks and grown under the control (Ct) conditions and with deficit irrigation (DI, 50% ETc) treatment determined from 2018 to 2021. Data are shown as the mean values for $n = 8$ plants. For each year, different letters indicate significant differences at $p < 0.05$ (LSD test); ns: not significant.

The TSS content showed a similar pattern to the AI (Figure 6B). Although no differences between the rootstocks were observed for the Ct conditions, as a general trend showed that the TSS increased with DI during all of the harvests, except for 2019. The CC DI obtained the highest value in 2018, followed by the FA-5 DI plants (20.7% and 10.3% increases related to Ct, respectively). The TSS parameter increased by around 21.6% and 12.0%, respectively, in both the DI rootstocks in 2020 and 2021 compared to the Ct plants.

The RI parameter (Figure 6C) was the least influenced by the rootstock or DI because similar values between the rootstock–treatment combinations were observed for three of the four harvests. Irrigation stress lowered the RI at the end of the experiment, but without differences between the rootstocks (17.9% mean reduction value).

3.4. Correlation Analysis

Correlation analyses were carried out to estimate the relation between the yield and fruit quality in the plants of both genotypes grown according to the two treatments (Table 4). The pairwise coefficients showed a positive correlation and statistical significance for 23 pairs of traits of the 209 studied ones, while negative relations were observed for 25 pairs. The most representative positive relations appeared among the yield, big diameters and the RI. The volume canopy was positively correlated with medium–large-sized fruits and the rag and juice contents, while fruits with high percentages of peels induced fruits with reduced rag and juice fractions and rich quality (high AI, TSS and RI). Small fruits presented high firmness and AI but low RI. Yield was negatively related to small fruits, which usually had high thickness and acidity levels, but a low RI.

Table 4. Correlation analysis between the parameters measured in 2021. Numbers in bold ** and * indicate significance at the $p < 0.01$ and $p < 0.05$ values for r. F: fruit; N: number; FW: fresh weight; D,Ø: diameter; H: height; AI: Acidity Index; TSS: total soluble sugars; RI: Ripening Index.

	Yield	F				Ø (mm)									%			AI	TSS	RI
		N	FW	D/H	Th	<46	46–50	50–54	54–58	58–62	62–66	66–70	70–74	>74	Peel	Rag	Juice			
Vc	0.80	-0.86	0.44	0.27	**−0.95 ***	−0.79	−0.93	−0.91	−0.73	0.89	**0.95 ***	0.81	0.78	0.45	**−0.99 ****	0.99 **	**0.99 ****	**−0.97 ***	**−0.96 ***	0.91
Yield		−0.67	0.71	0.15	**−0.94 ***	−0.88	−0.89	**−0.97 ***	**−0.96 ***	0.87	0.93	**0.99 ****	**0.99 ****	0.84	−0.76	0.74	0.78	−0.92	−0.81	**0.98 ***
F N			−0.71	−0.73	0.75	0.90	0.93	0.83	0.47	**−0.94 ***	−0.87	−0.74	−0.62	−0.16	0.87	−0.82	−0.89	0.85	**0.96 ***	−0.74
F FW				0.69	−0.54	−0.89	−0.75	−0.72	−0.51	0.80	0.69	0.79	0.68	0.40	−0.42	0.35	0.48	−0.62	−0.65	0.61
F D/H					−0.14	−0.61	−0.50	−0.32	0.13	0.56	0.36	0.28	0.09	−0.35	−0.31	0.23	0.36	−0.29	−0.51	0.16
F Th						0.83	0.93	**0.97 ***	0.91	−0.89	**−0.97 ***	−0.93	−0.93	−0.71	0.92	−0.92	−0.92	**0.97 ***	0.91	**−0.99 ****
Ø < 46							**0.96 ***	0.94	0.71	**−0.98 ***	−0.93	−0.93	−0.85	−0.50	0.78	−0.72	−0.81	0.89	0.91	−0.87
Ø 46–50								**0.97 ***	0.75	**−0.99 ****	**−0.97 ***	−0.92	−0.86	−0.49	0.92	−0.88	−0.94	**0.97 ***	0.98	−0.93
Ø 50–54									0.88	**−0.96 ***	**0.90**	**−0.98 ***	**−0.95 ***	−0.68	0.88	−0.86	−0.89	**0.99 ***	0.93	**−0.99 ***
Ø 54–58										−0.72	−0.84	−0.91	**−0.98 ***	−0.94	0.68	−0.68	−0.68	0.84	0.67	−0.94
Ø 58–62											**0.97 ***	0.92	0.84	0.47	−0.88	0.84	0.91	**−0.95 ***	**−0.97 ***	0.90
Ø 62–66												0.95 *	0.91	0.61	−0.93	0.90	0.94	**−0.99 ****	**−0.97 ***	**0.97 ***
Ø 66–70													**0.98**	0.77	−0.77	0.74	0.79	−0.93	−0.85	**0.96 ***
Ø 70–47														0.87	−0.74	0.72	0.75	−0.90	−0.78	**0.97 ***
Ø > 74															−0.39	0.39	0.38	−0.61	−0.38	0.76
%Peel																**−0.99 ****	**−0.99 ****	**0.95 ***	**0.96 ***	−0.88
%Rag																	0.99	−0.93	−0.93	0.87
%Juice																		**−0.96 ***	**−0.98 ***	0.89
AI																			**0.96 ***	**−0.98 ***
TSS																				−0.89
RI																				

4. Discussion

4.1. Effect of DI on Tree Development and Yield

For citrus trees under Mediterranean climate conditions, a lack of water is a limiting factor in fruit growth, FW and number, which determine the tree yield [14], as supported by the relations among Vc and FN in the correlation analysis. Other abiotic stresses, such as high carbonate content or salt conditions [16], can also lower these parameters in citrus trees, but can be ruled out in our work according to the soil analysis results. In our study, the trees grafted onto the CC and FA-5 rootstock selections underwent standard tree development, like those achieved by other authors in clementine and lemon [16,22,23], and no differences were found in Vc between the Ct rootstocks. A certain effect of variety should not be ruled out, as observed in the very early-season mandarin Orogros (smaller trees when grafted onto CC), but not in Clemenrubi [24]. The early satsuma Clauselina was smaller than Okitsu regardless of the rootstock [25].

As a general trend, water stress reduces the plant growth parameter because the trees with lower Vc are correlated more with a restricted water status and with fruit development and smaller diameters. Some authors have related this to a small leaf surface as an adaptive plant mechanism to prevent water loss due to transpiration under abiotic stress [21,26,27]. This assumption is partially fulfilled in our work. Although we did not observe any correlation between Vc and yield, the trees with the biggest diameters in fruit induced high yields. Other studies reported that the fruit FW and fruit number depend on the irrigation system because the highest yields (positive relation among yield, fruit FW and fruit number) have been observed for subterranean drip irrigation compared to the surface type [28]. Nules clementine trees submitted to 100–40% ETc presented a significantly reduced tree yield compared to normal irrigation, while 70% ETc DI affected the fruit FW, but not the fruit number, per tree. Hence, this irrigation practice barely reduced the tree yield [28]. When reducing water supply and increasing water deficit, a smaller tree yield was also found in Nules clementine [8]. Similarly, the authors of [28] observed a reduced tree yield when submitting clemenule trees to continuous DI throughout the vegetative cycle of these plants.

However, a reduced tree size is an interesting issue in citrus breeding programs as long as the yield efficiency is not affected, which also seems to be an interesting result of our study, because no significant differences were found in the mean fruit number and the mean yield per tree between the Ct and treated plants (Table 3). Similar results have been reported in trees grafted onto some FA hybrids, Cleopatra mandarin and *C. macrophylla* rootstocks [29,30]. This is also a point to favor semidwarfing materials like rootstocks FA-418 and FA-517. Some authors showed tree size reduction, but good yield efficiency, when combining semidwarfing materials with navel orange and lemon varieties [16,22,31,32]. Until the appearance of these materials, the availability of dwarfing rootstocks in citrus was scarce and restricted almost exclusively to Flying Dragon (*Poncirus trifoliata* L. Raf var. monstrosa [33]). However, this rootstock is susceptible to iron chlorosis, which limits its diffusion in commercial orchards [34]. So, FA-418 and FA-517 present clear advantages for use in orchards employed for intensive cultivation purposes.

The good tree size (Figure 1, Table 3) in the DI combinations in the first experiment years suggest that photosynthesis and carbohydrate distribution were not disturbed, at least not at this controlled DI level. A similar result was previously reported in lemon and orange trees [16,22,35]. In contrast, pomelos and mandarins were irrigated according to their water needs had small leaf sizes [36,37]. However, as the trees grew (the last 2 years of the experiment), the photoassimilate needs also grew and induced lower Vc in the DI plants (Figure 1). Although this assumption should be reinforced by gas exchange measurements in our experiment, the best results from the FA-5 DI plants suggests a higher basal-endogenous A_N value than CC, as supported by a previous bibliography [21]. This response of the plants treated by low-controlled irrigation has also been described in other crops as an adaptive response to water and salt stresses despite dehydration processes [38]. Moreover, the carbohydrate contents in leaves and, consequently, the source–sink imbalance strongly regulate CO_2, and sugar accumulation in the leaves is the signal that regulates the feedback mechanism to stimulate photosynthesis [39]. The DI-related reductions in A_N were much lower in FA-5 than in its parents (*C. macrophylla* and *Poncirus trifoliata* rootstocks [21]) and were related to hormonal regulation under water stress, especially abscisic acid induction in roots and leaves [40].

4.2. Effect of DI on Fruit Internal Quality

Like plant development, the fruit quality parameters can also be affected by abiotic stress, especially when the water supply is restricted. Of the studied traits, one physical (fruit diameter and its distribution, Figures 3A and 4) and two internal (AI and TSS, Figure 6A,B) parameters were the most influenced by water deficit from very early time points in the experiment, as also reflected by the correlation analysis (Table 4). In contrast, other traits were DI-dependent in the last study year, like % of peel (higher for DI, Figure 5B) and % of juice (lower for DI, Figure 5D), and likely depended on the plant water content and the predominant compounds in the cells of these fractions. The peel thickness (Figure 3C) was not affected by DI, which suggests that cell development in this fruit part is better able to counteract the low irrigation level than cells from the internal fruit part. This is supported by previous works conducted in the Nules clementine trees submitted to 100–40% ETc or 70% ETc DI regimes [28], grapefruit [41], Salustiana orange [42] and Satsuma mandarin [43]. Increments in the TSS and the AI were observed under the restricted water status, which was also recorded when submitting clemenules trees to continuous DI [30]. Higher AI values in clementine with decreasing water doses has also been reported [8]. However, DI 100–40% ETc generated fewer TSS and a lower AI in relation to 100% ETc, while 70% ETc did not show any differences with the Ct trees [28,44].

5. Conclusions

Considering the overall results of this study, we can conclude that with DI, the total production decreased, but with differences between the rootstocks. The reduction in CC production was mainly due to a smaller fruit size, as reflected by their lower average weight and their size distribution in some experimental years, which is a clear shift toward a smaller size. With FA-5, this reduction was due mostly to a smaller fruit number, and not to size, because it equaled, or was even bigger than, the Ct CC fruits.

Regarding fruit characteristics, the differences between the Ct and DI plants were bigger for the fruit on CC, which had a lower juice content (%), a higher rind percentage and equally higher TSS and AI values than CC Ct. For the fruit on FA-5, the differences between the Ct and DI trees were smaller, and the most remarkable effect was the increase in TSS with the DI treatment.

To conclude, although this is a 4-year study and more harvests are needed to corroborate FA-5 behavior, this work offers a good preliminary result to support using this rootstock in Orogrande mandarin under water stress.

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