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INTERNATIONAL **Agrophysics**
www.international-agrophysics.org

Int. Agrophys., 2018, 32, 325-333
doi: 10.1515/intag-2017-0018

Rootstock's and scion's impact on lemon quality in southeast Spain

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Received September 29, 2017; accepted February 21, 2018

A b s t r a c t. In terms of growth, yield and fruit quality, three lemon varieties ('Fino49' F49, 'Verna50' V50 and 'FinoElche' FE) were tested on three new hybrid root stock selections (Forner-Alcaide 2324, Forner-Alcaide 418 and Forner-Alcaide 5). Yield was weighed within a nine year period and fruit quality was determined in two harvests. Tree size was similar on the Forner-Alcaide 5 and Forner-Alcaide 2324 rootstocks, but reduced by 50% on Forner-Alcaide 418. Forner-Alcaide 2324 and the Forner-Alcaide 5 rootstocks showed similar cumulative yield, while it was decreased by 66% in Forner-Alcaide 418. In all cases, 'Verna50' was the less efficient scion. Rootstock was found to significantly affect fruit quality variables. The Forner-Alcaide 418 rootstock induced the lowest peel thickness and peel percentage, but the highest juice content and colour index. Forner-Alcaide 5, together with Forner-Alcaide 418, showed the highest total soluble sugars. In conclusion, both the Forner-Alcaide 5 and Forner-Alcaide 2324 rootstocks generate standard tree size and high yield in the varieties tested, together with great fruit quality, being a suitable alternative for replacing *C. macrophylla* rootstock when used in lemon varieties. Forner-Alcaide 418 reduces tree size without decreasing yield efficiency and improves alternate-bearing of the harvest. Thus, it may have use in intensifying citrus production as it behaves as a dwarfing rootstock.

K e y w o r d s: citrus rootstock, Forner-Alcaide, fruit quality, lemon, yield

INTRODUCTION

Spain is the fourth largest citrus producer in the world and one of the fresh-market citrus leaders, with more than 300 000 ha planted (MAGRAMA, 2014). Spain produces 700 t of lemons, mainly in the southern part of the Alicante and Murcia regions. In these areas, heavy soils and problems of salinity and iron chlorosis frequently appear and may limit the use of some citrus rootstocks.

Prior to the arrival of citrus tristeza virus (CTV) to Spain, sour orange (*Citrus aurantium* L.) was the standard rootstock in all plantations. Currently, almost 80% of citrus trees are budded onto Carrizo citrange (*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.), but this rootstock does not grow well in such soils. Thus, in lemon orchards, *C. macrophylla* Wester is the only usable rootstock. It is tolerant to salinity, iron chlorosis and *Phytophthora* spp., but is susceptible to CTV and cold and the fruit produced has a low quality. Therefore, other rootstocks are being considered (Castle, 2010).

In 1974, a programme was launched to breed citrus rootstocks by traditional hybridisations at the Valencian Institute for Agricultural Research (IVIA). One of its purposes was to obtain rootstocks that grew better in Spanish soil conditions than rootstocks currently used (Forner *et al.,* 2003). A specific aim of the programme was to develop rootstocks tolerant to salinity, iron chlorosis, water stress or flooding conditions. One of the results of this breeding programme is Forner-Alcaide 5 citrus rootstock (FA 5), a Cleopatra mandarin (*C. reshni* Hort. ex Tan.) x *P. trifoliata* hybrid, resistant to citrus tristeza virus (CTV) (Forner *et al.,* 2003), salinity (Forner-Giner *et al.,* 2009, 2011), iron chlorosis (Gonzalez-Mas *et al.,* 2009; Llosa *et al.,* 2009; Martinez-Cuenca *et al.,* 2013) as well as water stress (Rodríguez-Gamir *et al.,* 2010a,b), and gives good yield

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**This work was funded by National Institute of Agricultural and Food Research and Technology (RTA2014-00059), Valencian Generalitat and FEDER funds from 2015-2017.

and fruit quality to citrus scions (Forner-Giner *et al.,* 2003, 2009, 2011). Its behaviour with oranges and mandarins has also been studied (Forner-Giner *et al.,* 2003, 2009). Another outcome from the pro gramme is the FA 418 rootstock, a Troyer citrange (*C. sinensis* x *P. trifoliata*) x common mandarin (*C. deliciosa* Ten.) tolerant to CTV, which also presents dwarfing behaviour (Forner-Giner *et al.,* 2014). It also enables high yields and good fruit quality (Gonzalez-Mas *et al.,* 2009; Legua *et al.,* 2011b, 2013, 2014; Forner-Giner *et al.,* 2014). These two rootstocks together with other selections actually in registration process in Spain, such as FA 2324, enhance the agronomical behaviour of the actual rootstocks commonly used in Spain (Legua *et al.,* 2011b, 2014). Trees on the FA2324 rootstock, *i.e.* Troyer citrange x Cleopatra mandarin hybrid that is tolerant to CTV, salinity and iron chlorosis, with good fruit quality and good yields (Legua *et al.,* 2011b). Each rootstock is under EU protection.

The performance of these rootstocks grafted with lemons has been the subject of interest as a potential alternative to *C. macrophylla* in the area of Elche and south of Alicante. As regards lemon production, the most important lemon varieties in Spain are 'Verna' (13000 ha) and 'Fino' (23 000 ha) (MAGRAMA, 2014). 'Verna' is one of the main cultivars in Spain. Fruits are harvested from March to July, and despite its tendency towards alternate-bearing, they usually flower three or even four times. This contributed this variety's being cultivated on 13 000 ha in Spain (MAGRAMA, 2014). On the other hand, the 'Fino' variety produces lemons from October to February with high-quality fruits suitable for export to the rest of Europe. As it is the first maturing variety in Spain, it is grown mainly in valleys where late frosts are more likely to occur. 'Fino' accounts for about 23 000 ha of Spanish lemon production (MAGRAMA, 2014). Although 'Fino' fruits are smaller than that of 'Verna', they contain higher juice content percentage and acid level except at the very beginning of the season when the fruit is harvested before full maturity (Saunt, 2000). Recently, a local mutation of the Fino variety called 'Elche' is being planted in Spanish lemon zones. Field studies point to a good performance on poor soils.

The objective of this study was to evaluate the behaviour of three commercial varieties of lemon fruit budded onto several FA hybrids and grown on poor soils.

MATERIALS AND METHODS

The citrus rootstocks used in the experiment were FA 5, FA 2324 and FA 418. Seeds came from the original trees, and that of the parents from the mother seed trees held in the germplasm collection at IVIA (Instituto Valenciano de Investigaciones Agrarias). Scion response was studied on two common lemon varieties ('Fino' -F49- and 'Verna' -V50-), and on a local mutation of Fino called 'Elche' (FE). Buds of F49, FE and V50 were obtained from the germplasm collection at the IVIA. Rootstock seedlings were budded when they were 12 months old.

All nursery processes were performed in an aphid-proof greenhouse with a cooling system. Temperatures ranged between 18 and 27ºC and relative humidity was at about 80%. In April 1999, one year after budding, the nursery trees were planted in a randomised complete-block design with 5 single-tree replications for each scion/stock combination, and the tree spacing was 6 x 4 m. The plot was surrounded by border rows on all four sides.

The field was located at the IVIA Experimental Station in Elche (38º 14' 56.47'' N, 0º 41' 35.95'' W), a village near Alicante about 10 km from the Mediterranean Sea, Spain. The soil texture within the first 50 cm depth was classified as clay loam soil, with pH 8.5, $CaCO_3$ 44.4%, active calcium carbonate 17.1% and electric conductivity in the saturation extract at 25°C of 5.79 mS cm^{-1}.

Standard cultural practices were used for this variety, with drip irrigation and chemical weed-control. Water pH was 7.8, electrical conductivity ranged from 2.0 to 3.5 mS cm^{-1} mS cm^{-1} and 400 to 500 mg kg^{-1} of B. Fertilisation was applied from the 2nd year onwards and increased annually. Since the 6th year, the amounts applied 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 – 0.02 kg $tree^{-1}$. After 3 years of cultivation, trees were hand-pruned annually after harvest to get more regular and balanced production. Pruning consisted of removing unproductive, dry, misplaced or excessively long branches.

In January 2007, tree height, canopy diameter, trunk girth at 10 cm above and below the bud union were measured for all trees, and scion/stock ratio and trunk cross-sectional area (TCSA) were calculated. Canopy volume was calculated using Turrell's formula (1946).

Fruits were sampled when the first commercial harvest took place in nine consecutive years (from 2007 to 2015). Lemon samples were collected in early July for V50 fruits and early February for F49 and FE. Fruits from each tree were harvested, and included the collection of the recently dropped fruits so as to determine yield (kg $tree^{-1}$). To evaluate possible differences between rootstocks in terms of pre-harvest drop, No. 2,4-D treatments were applied and on the 7th, 8th and 9th harvests, the fruits dropped were weighed every two weeks. The weights of these fruits were added to the weights of harvested fruit. Cumulative yield (kg $tree^{-1}$) for the nine-year period was also calculated and yield efficiency (kg m^{-3}) was estimated as the ratio of cumulative yield to canopy volume. The alternate-bearing index (ABI) was ascertained by dividing the difference between two consecutive harvests by the sum of two yields × 100% for the nine harvests. If the index is more than 50%, the tree is alternate-bearing. If this index is less than 50%, the tree is regular-bearing.

Fruit quality was determined for the 2013/14 and 2014/15 harvests. At the harvest date, a selection of representative fruits reaching maturity was randomly collected from 5 trees – 20 fruit per tree (5 fruit from each cardinal canopy position; a total of 100 fruit of each scion/stock combination).

One sub-sample of 25 fruits of each scion/stock combination was selected to measure fruit physical and chemical characteristics. Fruit weight was measured with a digital balance (Sartorius, Model BL-600, 0.01 g accuracy). Fruit diameter (D), fruit height (H) and peel thickness were measured with an electronic digital slide gauge (Mitutoyo, Model CD-15 DC, 0.01 mm accuracy) and fruit shape index (D/H) was calculated. Colour determination was made for fruit skin on four opposite faces in the equatorial zone. The CIELAB L* (brightness or lightness; 0 = black, 100 = white), a* (–a* = greenness, +a* = redness) and b* (–b* = blueness, +b* = yellowness) colour variables were measured using a chromatometer (Minolta, Model CR-300, Ramsey, NJ) coupled to a Minolta DP-301 data processor. Colour index (CI) was then calculated using the formula $CI = 10^3 a^*/L^*b^*$ (Jimenez-Cuesta *et al.,* 1981). Fruit firmness was measured on the equatorial surface of the fruits by using a flat steel plate coupled with a texturometer (TAXT2i Texture Analyser, Stable Microsystems, Godalwing, UK) interfaced to a personal computer (Legua *et al.,* 2013). A bevelled holder prevented the bruising of the opposite side. Puncture resistance was measured with a 10 mm cylindrical probe fitted to the texture analyser. Lemon was placed upon a flat plate and the stem calyx axis was made parallel to the plate. The test was carried out at the probe speed of 2 mm s^{-1}. The results were expressed as the force to puncture resistance (kg cm^{-2}).

The rest of each sample was divided into three sub-samples (25 fruits each) and used to determine quality characteristics in the juice. Fruits were squeezed together (3 replicates for each scion/stock combination) with an electric squeezer and the peel, pulp and juice percentages (w/w) were calculated. Juice pH was measured with a pH-meter (Crison, Model micropH 2001) and colour index was measured as described previously for fruit skin. Total soluble solids (TSS) was measured with a refractometer (Model N-1, Atago (±0.2ºBrix)) and expressed as degrees Brix at 20ºC. The method for the analysis of titratable acidity (TA) was based on neutralisation (NaOH 0.1 N) to pH 8.1 and values were expressed as g l^{-1} citric acid. The ripeness index (RI) was calculated as the TSS/TA ratio.

Data were subjected to ANOVA to test for significant differences between treatments. Before carrying out any statistical analysis, the normality of all the data was studied using the Kolmogorov-Smirnov test. In case the normality hypothesis was discarded at the 95% confidence level, the data were transformed according to the logarithmic function. Otherwise, the data analyses were carried out with the variables measured in their natural scales. The variance of the transformed or non-transformed data was partitioned through a variance analysis (ANOVA, Statgraphics Centurion for Windows, Statistical Graphics Corp.) into one source of variability. The experiment consisted of two factors: i) rootstocks, ii) varieties. The significance of the comparisons between treatments was analysed using Fisher's least significant difference (LSD) test at $p < 0.05$.

RESULTS

There were significant differences between rootstocks with regard to canopy diameter and tree height, but not between varieties or the interaction of both variables. Trees on FA 5 and FA 2324 did not present statistical differences in tree height (between 2.42 to 2.57 m) and canopy diameter (between 3.81 to 4.00 m), which reached a standard tree size for Mediterranean conditions (Table 1). However, the FA 418 rootstock reduced the canopy diameter and height of the trees by 45 and 50%, respectively. This suggests that FA 418 behaved as a dwarfing rootstock.

It was found that canopy volume was significantly influenced by the rootstock, variety and their interaction. While FA 2324 did not have influence on the canopy volume measured in any of the varieties tested, FE and V50 varieties grafted onto FA 5 reduced their canopy volume when compared with that of the F49 variety. Finally, although the

Table 1. Canopy diameter, tree height and canopy volume of three lemon varieties ('Fino' F49, 'Elche' FE and 'Verna' V50) on three Forner-Alcaide (FA) citrus rootstocks (FA 2324, FA 418 and FA 5)

Rootstock	Variety	Canopy diameter (m)	Tree height (m)	Canopy volume (m^3)
FA 2324	F49	3.9a	2.6a	19.9ab
	FE	4.0a	2.5a	20.9a
	V50	3.9a	2.6a	20.6a
FA 418	F49	2.1b	1.2b	2.8c
	FE	2.1b	1.3b	3.0c
	V50	2.2b	1.3b	3.5c
FA 5	F49	4.0a	2.5a	21.0a
	FE	3.9a	2.4a	19.2b
	V50	3.8a	2.4a	18.2b
Analysis of variance				
Rootstock		***	***	***
Variety		ns	ns	*
Rootstock x Variety		ns	ns	**

Mean separation within columns by the LSD test ($p < 0.05$). In each column, values with the same letter are not significantly different.

Table 2. Trunk cross-sectional area (TCSA), yield efficiency, scion/stock ratio and alternate-bearing index (ABI) of three lemon varieties (‘Fino’ F49, ‘Elche’ FE and ‘Verna’ V50) on three ‘Forner-Alcaide’ (FA) citrus rootstocks (FA 2324, FA 418 and FA 5)

Rootstock	Variety	TCSA (cm^2)	Yield efficiency ($kg\ cm^{-2}$)	Scion/stock ratio	ABI (%)
FA 2324	F49	317.94c	2.77 ab	0.86 ab	35.08 bc
	FE	414.65ab	2.19 abc	0.77 b	41.99 b
	V50	461.17a	1.31 c	0.87 ab	64.94 a
FA 418	F49	87.45d	2.73 ab	0.79 b	9.49 d
	FE	112.71d	3.10 a	0.96 a	14.61 d
	V50	118.94d	1.72 bc	0.84 b	22.84 cd
FA 5	F49	335.01 bc	3.02 a	0.86 ab	41.26 b
	FE	327.98 bc	2.52 ab	0.78 b	42.12 b
	V50	276.65 c	2.25 abc	0.85 b	61.80 a
Analysis of variance					
Rootstock		***	ns	ns	***
Variety		ns	**	ns	***
Rootstock x Variety		ns	ns	**	ns

Explanations as in Table 1.

canopy volume of the three varieties grafted onto FA 418 was similar, it was decreased to 85% when compared with that of the other two rootstock selections.

We also found differences between rootstocks in trunk cross-sectional area (TCSA, Table 2) but not between varieties or their interaction. While TCSA values were similar between varieties on the FA 418 or FA5 rootstocks, it was 27.4% lower in F49/FA 2324 trees than in other FA 2324 combinations. FE/FA 2324 and V50/FA 2324 trees showed the highest TCSA values, while the lowest were observed in FA 418 combinations. Furthermore, the scion/stock ratio was significantly influenced by the interaction of the rootstock and the variety (Table 2). While all FA 2324 and FA 5 combinations presented similar scion/stock ratio (which ranged from 0.77 to 0.87), this behaviour differed when grafted on the FA 418 rootstock. Thus, FE/FA 418 trees showed higher scion/stock ratio (17.8% increase) than the other varieties on the FA 418 rootstock.

The alternate-bearing index (ABI) differed between rootstocks and varieties, but no differences were found in the interaction (Table 2). Still, the rootstock showed clear influences on the ABI behaviour of the variety. While the FA 5 and FA 2324 rootstocks exhibited significant differences in this parameter, FA 418 did not. Interestingly, all trees on the FA 418 rootstock presented lower ABI values than on the rest of rootstocks. In addition, between varieties, V50 presented higher ABI values than the rest, which reflects an alternate-bearing behaviour in this lemon scion.

We also found significant differences in yield between the rootstocks, the varieties and the interaction between both factors (Table 3). The FA 2324 and FA 5 rootstocks showed similar cumulative yield (ranging from 766 to 783 kg $tree^{-1}$) and little differences between harvests. Cumulative yield in FA 418 was 66% lower than that of the rest of rootstocks. We also observed significant differences between varieties. Thus, harvests from the V50 scion were lower (35% decrease) than that from the F49 and FE trees. F49 and FE varieties showed the highest productivity (around 680 kg $tree^{-1}$) a 35% rise when compared with that of V50.

Despite the differences in yield described previously, yield efficiency was similar between rootstock selections. However, this parameter was significantly affected by the variety. Thus, according to this parameter, V50 was the less efficient scion from all the tested options and no differences were found between F49 and FE varieties (Table 2).

Fruit weight was also affected by the rootstock and the variety (Table 4). Fruits from trees grafted onto the FA 418 rootstock showed the lowest size when compared with fruits grafted on the rest of the selections. Other external size parameters such as fruit diameter, height and shape index strongly corresponded with fruit weight behaviour. Fruits from FA 418 also presented the lowest peel thickness and peel percentage. In contrast, juice content in these fruits was high and did not differ significantly from the juice from fruits grafted onto FA 5. Between varieties, the highest juice percentages was registered in fruits from the

Table 3. Yield and cumulative yield calculated from 2007 to 2015 of three lemon varieties ('Fino' F49, 'Elche' FE and 'Verna' V50) on three Forner-Alcaide (FA) citrus rootstocks (FA 2324, FA 418 and FA 5)

Rootstock	Yield (kg tree^{-1})									Cumulative yield (kg tree^{-1})
	2007	2008	2009	2010	2011	2012	2013	2014	2015	
					Rootstock					
FA 2324	6.21 a	15.80 b	56.75 a	134.04 a	78.87 b	87.33 a	160.42 a	154.00 a	72.08 a	765.53 a
FA 418	2.64 a	6.52 c	21.25 b	38.04 c	30.45 c	29.54 b	52.75 b	54.00 b	20.25 b	255.47 b
FA 5	5.25 a	23.47 a	53.79 a	113.00 b	92.45 a	96.46 a	168.0 a	168.33 a	62.41 a	783.21 a
					Variety					
F49	6.35 a	19.89 a	47.45 a	111.08 a	98.00 a	84.29 a	122.50 a	122.83 a	72.17 a	684.60 a
FE	3.26 a	16.32 ab	48.62 a	101.12 a	97.75 a	84.05 a	134.33 a	126.17 a	66.33 a	677.99 a
V50	4.48 a	9.59 b	35.70 b	72.87 b	6.04 b	45.00 b	124.33 a	127.33 a	16.25 b	441.62 b
					Analysis of variance					
Rootstock	ns	***	***	***	***	***	***	***	***	***
Variety	ns	ns	**	***	***	***	ns	ns	***	***
Rootstock x Variety	ns	ns	ns	ns	***	ns	ns	ns	***	***

Explanations as in Table 1.

Table 4. Fruit quality parameters (weight, diameter, height, shape index, peel thickness, peel, pulp and juice percentages, as well as fruit firmness) of three lemon varieties ('Fino' F49, 'Elche' FE and 'Verna' V50), on three Forner-Alcaide (FA) citrus rootstocks (FA 2324, FA 418 and FA 5)

Rootstock	Variety	Fruit weight (g)	Fruit diameter (cm)	Fruit height (cm)	Shape index (D/H)	Peel thickness (mm)	Peel (%)	Rag (%)	Juice (%)	Fruit firmness (kg cm^{-2})
FA 2324	F49	176.25 bc	68.58 cd	84.43 bc	0.82 c	7.12 ab	48.71 cd	16.09 bc	35.19 b	8.95 de
	FE	212.98 a	72.51 a	86.86 b	0.84 bc	7.64 a	53.87 b	11.86 d	34.26 b	11.23 cd
	V50	157.33 d	63.28 e	91.65 a	0.69 e	7.07 ab	56.60 a	13.19 d	30.21 c	16.20 a
FA 418	F49	179.52 bc	68.24 cd	83.46 ef	0.83 c	5.52 c	44.63 f	16.80 b	38.57 a	9.44 de
	FE	165.11 cd	66.66 d	80.76 f	0.84 c	4.79 d	46.27 ef	13.72 cd	40.00 a	8.79 e
	V50	123.92 e	59.30 f	81.21 f	0.76 d	5.36 cd	49.40 c	20.61 a	29.98 c	14.78 ab
FA 5	F49	184.25 b	70.19 bc	81.52 cd	0.86 ab	7.39 ab	48.64 cd	12.87 d	38.49 a	8.49 e
	FE	211.08 a	72.29 ab	82.41 c	0.88 a	6.80 b	47.06 de	13.81 cd	39.13 a	10.52 de
	V50	130.61 e	60.01 f	79.91 d	0.75 d	5.79 c	49.11 cd	19.60 a	31.28 c	12.97 bc
					Analysis of variance					
Rootstock		***	***	***	***	***	***	***	***	ns
Variety		***	***	ns	***	**	***	***	***	***
Rootstock x Variety		***	***	***	**	***	***	***	ns	ns

D – fruit diameter, H – fruit height. Other explanations as in Table 1.

Table 5. Peel and juice colour index (CI) of three lemon varieties ('Fino' F49, 'Elche' FE and 'Verna' V50) on three 'Forner-Alcaide' (FA) citrus rootstocks (FA 2324, FA 418 and FA 5). CI was calculated with the following formula CI = 10^3 a*/L*b* (Jimenez-Cuesta *et al.*, 1981), where colour variables were L* (brightness or lightness; 0 = black, 100 = white), a* (–a* = greenness, +a* = redness) and b* (–b* = blueness, +b* = yellowness)

Rootstock	Variety	Peel				Juice			
		L*	a*	b*	CI	L*	a*	b*	CI
FA 2324	F49	76.12 e	-9.83 bcd	62.77 d	-2.07 de	32.60 d	-2.81 cde	10.14 b	-8.69 b
	FE	78.38 cd	-10.10 cde	65.09 c	-1.99 de	32.20 d	-1.41 a	10.42 b	-4.22 a
	V50	77.88 d	-10.86 f	62.40 d	-2.28 f	35.29 a	-3.36 e	7.79 d	-12.33 c
FA 418	F49	78.68 bc	-9.40 ab	68.73 b	-1.76 ab	32.88 cd	-3.37 e	10.03 b	-10.23 b
	FE	78.74 abc	-9.05 a	70.56 a	-1.67 a	33.91 bc	-2.00 ab	11.25 a	-5.27 a
	V50	78.28 cd	-10.00 cd	65.66 c	-1.96 cd	33.66 bc	-2.49 bc	8.68 c	-8.66 b
FA 5	F49	79.06 ab	-9.63 bc	67.25 b	-1.83 bc	33.78 bc	-2.65 cd	9.14 c	-8.60 b
	FE	78.87 abc	-10.33 de	65.53 c	-2.02 d	32.17 d	-3.17 de	10.54 b	-9.38 b
	V50	79.40 a	-10.57 ef	62.40 d	-2.22 ef	34.10 b	-2.79 cde	8.83 c	-9.47 b
Analysis of variance									
Rootstock		***	***	***	***	ns	ns	ns	ns
Variety		***	***	***	***	***	***	***	***
Rootstock x Variety		***	ns	***	ns	***	***	**	***

Explanations as in Table 1.

Table 6. Quality parameters from juice of t lemon varieties ('Fino' F49, 'Elche' FE and 'Verna' V50) on three Forner-Alcaide (FA) citrus rootstocks (FA 2324, FA 418 and FA 5)

Variety	pH	TA (g l^{-1} citric acid)	TSS (º Brix)	RI
FA 2324				
F49	2.94	5.72	7.70 abc	1.35
FE	2.96	5.73	6.97 d	1.22
V50	2.95	5.47	6.67 d	1.23
FA 418				
F49	3.03	5.51	7.63 bc	1.39
FE	2.99	5.98	8.03 a	1.35
V50	3.01	5.39	7.53 c	1.40
FA 5				
F49	2.90	6.10	7.96 ab	1.32
FE	2.95	5.62	7.80 abc	1.39
V50	3.03	5.08	6.67 d	1.32
Analysis of variance				
Rootstock	ns	ns	***	ns
Variety	ns	ns	***	ns
Rootstock x Variety	ns	ns	***	ns

Explanations as in Table 1.

F49 and FE scions. In all cases, this parameter in the V50 scion was reduced between 14 and 23% when compared to the rest of varieties. Fruit firmness was only affected by the variety. Interestingly, the highest values were observed in the V50 scions.

Peel colour was significantly affected by the rootstock and the variety (Table 5). Fruits from FA 418 showed the highest CI, while, between varieties, the V50 fruits from all rootstocks presented the lowest CI values. However, juice colour index was not affected by the rootstock, and significant differences were only found when comparing the variety and the RxV interaction.

Regarding some variables of internal quality in the fruits such as pH, total acids and the ripeness index (RI) were not affected by the rootstock or the variety (Table 6). However, statistical differences were recorded in total soluble sugars (TSS) when analysed in all studied factors. Thus, FA 5, together with FA 418, showed the highest TSS values, while the lowest results between varieties were registered in the V50 fruits.

DISCUSSION

It is generally assumed that the rootstock greatly affects tree size (Bassal, 2009; Georgiou, 2002, 2009; Towokorski and Fazio, 2016), and was noted in scions grown upon citrus rootstocks such as 'Flying dragon' (Da Silva *et al.,* 2013; Martínez-Alcantara *et al.,* 2013). This effect is of notable interest in citrus breeding work as it reduces yield costs, which is one of the main objectives of our programme. In this study, trees grafted onto the FA 5 and FA 2324 rootstock selections acquired a standard tree development (Table 1) similar to that obtained in trees grafted onto 'Carrizo' rootstock (Georgiu, 2002). However, the FA 418 selection significantly reduced the size (height and canopy diameter and volume) of the tree (Table 1), as previously reported in other studies (Forner-Giner *et al.,* 2014). What is more, FA 418 also presented the lowest TCSA parameter, which is usually considered to be highly correlated with tree weight and canopy volume (Westwood and Roberts, 1970; Yildirim *et al.,* 2010).

Another good parameter in evaluating plant development and relative growth between both tree fractions is scion/stock ratio. This effect corresponds to the ratio of the circumference of the scion to that of the rootstock, and it reflects the difference in the growth rate of each tree fraction (Roose *et al.,* 1989). In our study, FA 418 had a growth rate similar to that of the FE scion (the mutation widespread in Alicante), whereas FA 2324 and FA 5 tended to grow more rapidly than did the scion. This factor is interesting as it reveals an acceptable agronomical behaviour in this combination that is similar to that of *C. macrophylla* (Georgiu, 2009; Perez-Perez *et al.,* 2005), the most often used lemon rootstock in Spain.

Tree yield is strongly influenced by the rootstock – as previously described in several studies carried out in lemon. Thus, *C. macrohylla* generates high crops in most lemon varieties, while other rootstocks like 'Carrizo citrange' or 'Cleopatra mandarin' induce low yields (Al-Jaleel *et al.,* 2005; Perez-Perez *et al.,* 2005; Georgiu, 2009). This effect is likely linked to their low TCSA (Yildirim *et al.,* 2010). Conversely, Figueiredo *et al.* (2005) observed good yields in 'Eureka lemon' on 'Cleopatra mandarin' rootstock. In our study, yield in FA 418 trees is lower than that in trees grafted onto the other two rootstocks (Table 3), which did not show differences between them. However, when we compare yield efficiency, we can observe that this parameter in FA 418 was similar to the value registered in the other two rootstocks (Table 2). Hence, although trees grafted onto FA 418 are smaller in size, they are as productive as the ones of standard size - as previously pointed by Forner-Giner *et al.* (2014). Perez-Perez *et al.* (2005) also observed good yield efficiency and small TCSA in 'Eureka' and 'Lisbon' trees grafted on *C. macrophylla* rootstock. Finally, it also suggests that photosynthesis and carbohydrate distribution are not disturbed in FA 418 combinations, despite their reduced tree size, as previously reported in other FA hybrids (Jover *et al.,* 2012).

The analysis of the alternate-bearing index (ABI) reflects the differences in yield along the harvest period of the trees, also shows important new information. Verna variety develops a very alternate behaviour, which was already observed in other studies (Blanco *et al.,* 1989). The FA 418 is a rootstock which reduces to a greater extent the alternate-bearing character even in the V50 variety. The low alternate effect of FA 418 was already observed in other varieties (Forner-Giner *et al*., 2014). Furthermore, some authors state that there are some rootstocks which increase alternate-bearing of the yield (Legua *et al.,* 2011b), with a harmful effect on those commercial varieties in which this defect is already present. The FA 5 and FA 2324 rootstocks did not behave as alternate when used over the F49 and FE scions , as previously observed in Lane Late orange (Legua *et al.,* 2011a), but they did when used over the V50 variety (Table 2).

Regarding external and internal fruit quality, major differences between rootstocks were registered with regard to important quality parameters such as fruit size, peel thickness and content, as well as juice and sugar content (Forner-Giner *et al.,* 2003, 2010, 2011; Legua *et al.,* 2011a,b) (Table 4). Fruit size is one of the main parameters for fresh market and it is well-known that rootstock exerts a great influence on it (Forner-Giner *et al.,* 2003, 2010, 2011). In general terms, all studied rootstocks increase the size of fruits of the 'Fino' and 'Elche' varieties, while 'Verna' fruits were the smallest in all cases. Worth highlighting is that the dwarfing rootstock FA 418 maintain good fruit growth in lemon fruits, as previously observed for orange varieties grafted onto this selection (Forner-Giner *et al.,* 2014). In other studies, trees on *C. macrophylla*, 'Volkamer lemon' and other less known rootstocks in Spain (*C. sulcata*, *C. taiwanica* and *C. ampullacea*) generate big lemon fruits (Al-Jaleel *et al.,* 2005; Yildirim *et al.,* 2010). In contrast, size reduction in lemon was observed in fruits from the Cyprus local variety 'Lapitkiotiki' on 'Cleopatra mandarin', Sour orange' and 'Morton citrange' rootstocks (Georgiu, 2009). *C. amblycarpa* and 'Cleopatra mandarin' rootstocks also induce a low fruit diameter in 'Eureka' lemon (Al-Jaleel *et al*., 2005). It was also observed that the choice of rootstock had a significant influence on some fruit parameters such as peel colour (Table 5). This was also observed by other authors in lemons and other citrus fruits (Perez-Perez *et al.,* 2005; Legua *et al.,* 2011b, 2013).

Other determinants of citrus fruit fresh quality are peel thickness, firmness or texture. Extremes in rind thickness are not desirable, as thick rind is normally related with low juice content, while thin rinds are prone to splitting and are sensitive to peel disorders which can occur during storage. In our study, fruits on FA 2324 show enhanced peel thickness and even generated a reduction of juice content in the F49 scion (Table 4). Peel thickness in varieties grafted onto FA 2324 was higher than in the rest of rootstocks, contrary to other studies carried out in 'Lane late'/FA 2324 trees (Legua *et al.,* 2011a).

Finally, the flavour and palatability of citrus fruit varies according to relative levels of TSS, and the presence or absence of aromatic or bitter juice constituents (Davies and Albrigo, 1994). Although fruit quality standards for lemon juice do not include a minimum requirement for soluble solids concentration, this parameter should not be ignored. In general terms, in citrus fruits, the larger the size and the thicker the rind, the lower its juice content. This is the case of the higher juice content of 'Marsh' grapefruit on 'Sour orange' than on 'Amblycarpa' or 'Cleopatra mandarin' (Economides and Gregoriu, 1993). In our study, although the ripeness index was apparently unaffected (Table 6), statistical differences were observed in sugar content clearly influenced by the rootstock and the variety (Table 6). This effect is very important in orange and mandarin fruits (Georgiou, 2009; Legua *et al.,* 2014), and it has also been observed in other studies carried out on lemons (Al-Jaleel *et al.,* 2005; Perez-Perez *et al.,* 2005). Thus, Misra *et al.* (1999) obtained the maximum juice content in fruits from lemon trees on 'trifoliate orange' and 'Cleopatra mandarin', while the lowest was found on *C. taiwanica* (Zekri and Al-Jaleel, 2004). However, not all studies demonstrated rootstock's influence on juice content. For example, no significant differences in juice content of 'Comune Clementine', 'Orlando tangelo', and grapefruit were found from trees grown on all the studied rootstocks (Continella *et al.,* 1988; Fallahi and Rodney, 1992; Wutscher *et al.,* 1975).

CONCLUSIONS

1. Citrus rootstock FA 418 reduces tree size in the three lemon scions studied, and behaves as a dwarfing rootstock for these varieties.

2. Citrus rootstock FA 418 did not decrease the yield efficiency of the trees and it reduced the alternate-bearing of the harvest when compared with the other studied selections.

3. Both the FA 5 and FA 2324 rootstocks generate standard tree size and high yield, together with great fruit quality, in the varieties tested. The aforementioned should, therefore, be considered suitable alternatives for replacing *C. macrophylla* rootstock when used in lemon varieties.

Conflict of interest: The Authors do not declare conflict of interest.

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