

# **Influence of Rootstock on Citrus Tree Growth: Effects on Photosynthesis and Carbohydrate Distribution, Plant Size, Yield, Fruit Quality, and Dwarfing Genotypes**

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## **Abstract**

Citrus species are the most widely produced fruit crops in the world, and Spain is one of the leading citrus producers that supply the fresh market. Rootstocks greatly influence variety behaviour as it ensures tolerance to abiotic stress conditions, as well as the provision of minerals and water for the total plant, and consequently impact crop yield and fruit quality. So, rootstock choice is one of the most important decisions a grower makes when establishing commercial citrus orchards. In this chapter, we attempted to provide an overview of the response in terms of plant growth, fruit quality and yield parameters of several citrus cultivar trees grafted onto different commercial rootstocks, plus new hybrids and some dwarfing genotypes, to reduce costs in some cultural practices. In particular, we considered the rootstock influence on scion photosynthetic capacity linked to carbohydrate distribution for plant vegetative and reproductive development.

**Keywords:** breeding, citrus, drought, dwarfing, Fe chlorosis, flooding, plant growth, rootstock, salinity, yield

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## **1. Introduction**

Spain is one of the leading citrus producers that supply the fresh market worldwide. There is a huge variety of cultivars that gives rise to vigorous trees, produces high-quality fruit and

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allows to extend the commercial period for these fruits from September (earlier clementines, *Citrus reticulata* Blanco) to May (late oranges, *Citrus sinensis* L.). However, several environmental factors could threaten the citrus industry.

The main factors that limit citrus growth include Citrus tristeza virus (CTV) and *Phytophthora* spp., which are present in almost all Spanish citrus orchards. Some abiotic stresses, such as salinity and flooding, also reduce citrus growth in different citrus areas. Moreover, much soil is calcareous and frequently contains over 30% CaCO<sub>3</sub> with pH values between 7.5 and 8.5, which causes Fe-deficiency in plants. For these reasons, the trees grown on the rootstocks currently used in Spain face certain problems. Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], currently the most important rootstock used in Spain, is susceptible to salinity and lime-induced chlorosis. Cleopatra mandarin (*C. reshni* Hort. ex. Tan.) is tolerant to these problems, but tends to grow slowly for the first few years after planting, and induces low yield and fruit size in some varieties. Trees on *C. volkameriana* Ten. & Pasq. are vigorous and bear precociously, but are more susceptible to *Phytophthora*. Thus, attempts have been made to solve abiotic problems through citrus-rootstock breeding programmes worldwide.

Choice of rootstock is among the most important decisions a grower makes, and implications for yield and quality are enormous. Drivers of rootstock adoption are wide-ranging with the most important being tolerance to CTV, *Phytophthora*, nematode and salt, but water-use efficiency and drought tolerance are increasingly becoming important to achieve better performance (Table 1). Although the metabolic functions in a grafted plant are divided

	Carrizo citrange	Swingle citrumelo	Cleopatra mandarin	<i>Citrus</i> <i>volkameriana</i>	FA 5	FA 13	FA 517	FA 418
<b>Pests and diseases</b>								
Tristeza	R	T	T	T	R	R	R	T
Phytophthora	••••	•••••	•••	•	••••	•••	••••	•
Nema	S	R	S	S	R	S	R	S
<b>Abiotic stress</b>								
Salinity	•	•••	••••	•••	•••••	•••••	••••	•••
Clay soil	••	•	••••	••••	•••	••	••••	••
Flooding	•••	•••••	•	•••	••••	••••		
<b>Horticultural traits</b>								
Tree size	••••	•••••	••••	••••	••••	•••	••	•
Yield	•••	•••	••	••••	••••	••••	••••	••
Fruit size	•••	••	••	••••	•••	•••	•••	•••
Maturity	••••	••	•••	••••	••••	••••	••••	•••

Symbols key: good/high (•••••) to low/poor (•), resistant (R), susceptible (S), tolerant (T).

**Table 1.** Tolerance average behaviour of main Citrus rootstocks used in Spain to main pests and diseases and abiotic stress factors influencing on plant growth. Effect of citrus rootstock on several horticultural traits related to tree yield and fruit quality.

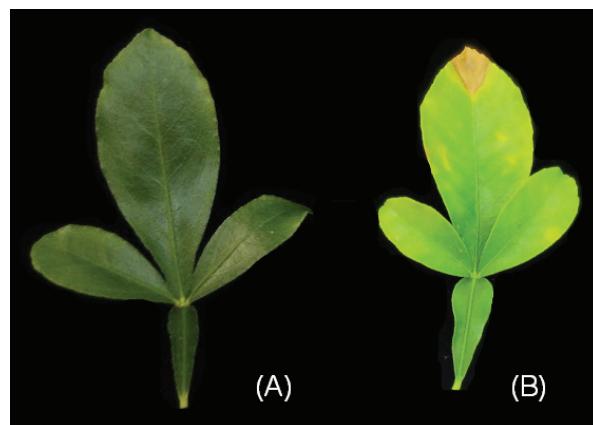
between the two plant fractions, it is well known that rootstocks greatly influence variety behaviour as it ensures provision of minerals and water for the total plant.

This work overviews the response in terms of plant growth, fruit quality and yield parameters of several citrus cultivars trees grafted onto different commercial rootstocks, plus new hybrids grown under field conditions. The use of some dwarfing genotypes, in which small tree size helps to reduce costs in some cultural practices such as pruning and harvesting, was also considered. This work also reveals the relation between gas exchange parameters, carbohydrate distribution during the annual cycle or hydraulic conductance in roots, shoots and graft union segments as pivotal factors for regulating vegetative and reproductive development. In conclusion, the influence of rootstocks on scions photosynthetic capacity may be a key consideration when determining citrus plant performance in terms of vigour, crop load and fruit characteristics.

## 2. Factors that limit growth in citrus orchards

### 2.1. Salinity

Citrus are frequently cultivated in semiarid areas where many soils are either affected by salt or present a high salinisation risk. Soils are considered saline when ECe (electrical conductivity of soil saturated paste extract) is  $4 \text{ dS m}^{-1}$  or more, which is approximately the equivalent to  $40 \text{ mM NaCl}$  and generates an osmotic pressure of about  $0.2 \text{ MPa}$  [1]. Citrus is considered a salt-sensitive crop [2], which suffers physiological disturbances and growth reduction even at low to moderate salinities (**Figure 1**).



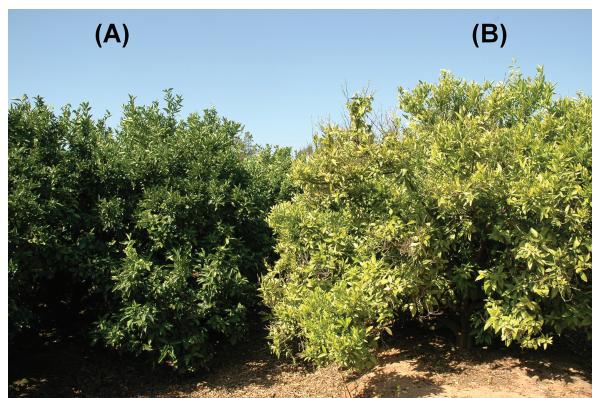
**Figure 1.** Carrizo Citrange leaves affected by salinity stress. (A) Control; (B) salt stressed symptoms (leaf yellowing and tip burns).

Salinity reduces growth and causes physiological disorders [3, 4], partly induced by adverse water relations due to a reduced soil solution osmotic potential, which alters leaf gas exchange parameters. Salt stress has been shown to decrease water potential ( $\psi_w$ ), stomatal conductance (gs), transpiration (E) and net CO<sub>2</sub> assimilation rates ( $A_{CO_2}$ ) in leaves [5–7]. Excessively high concentrations of saline ions in leaves cause specific toxicities and nutrient imbalance [6, 8].

Major differences in salt stress tolerance have been found between species and family members. Uptake and/or transport of saline ions to the scion is controlled by the rootstock, which chiefly determines chloride (Cl<sup>-</sup>) and sodium (Na<sup>+</sup>) accumulation in leaves [6, 9–11]. Since the main ion that causes damage is Cl<sup>-</sup> [12, 13], the salt tolerance of some citrus rootstocks is usually established by their capacity to exclude Cl<sup>-</sup> from leaves [5]. In addition, tolerance to other salt-related stresses, like B toxicity, is likely based on the down-regulation of the main B transport channels in the root, *NIP5* and *PIP1* genes, the capacity to hold B in an insoluble form in the leaves mainly allocated in cell walls, and compartmentalisation of toxic B from the cytoplasm inside the vacuole due to the up-regulation of aquaporin TIP5 [14–16].

## 2.2. Fe deficiency

It is estimated that between 20% and 50% of the fruit trees grown in the Mediterranean basin suffer from iron (Fe) deficiency [17]. The most prevalent cause of Fe deficiency in this region is the presence of high levels of carbonate ions in calcareous soils, which lead to a high pH, low Fe availability and the condition known as lime-induced chlorosis [18]. The citrus trees planted in these soils often show signs of severe Fe deficiency or Fe chlorosis because of low Fe availability. Iron deficiency affects the biochemistry, morphology and physiology of the whole plant because Fe is an important cofactor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls, which turns into impaired plant growth [19, 20] (Figure 2).



**Figure 2.** Citrus tree affected by iron deficiency at field conditions. (A) No symptoms; (B) Fe-chlorotic symptoms (yellowing) and defoliation branches.

Under Fe-deficient conditions, citrus, like other dicots, have developed the Strategy I mechanism to increase Fe uptake capacity in the root system, which includes increased rhizosphere acidification and  $\text{Fe}^{3+}$  reduction through proton-ATPases ( $\text{H}^+$ -ATPase) and ferric chelate reductase (FC-R) enzymes, respectively, and stimulation of  $\text{Fe}^{2+}$  transport across root cell membranes mediated by a specific iron-regulated transporter, IRT [21]. In the xylem,  $\text{Fe}^{3+}$  is transported to leaves chelated by low-molecular-weight organic acids, mainly citrate and malate; which accumulate in leaves, xylem and roots in response to Fe deficiency [22].

Similar to other disorders, citrus-growing success under iron chlorosis conditions, such as calcareous soils, depends on the availability of suitable rootstocks that are tolerant to low Fe. Trifoliolate orange [*Poncirus trifoliata* (L.) Raf.], sweet orange [*C. sinensis* (L.) Osb.] and Carrizo citrange are all susceptible to lime-induced chlorosis, whereas sour orange (*C. aurantium* L.), Cleopatra mandarin and FA 5 (*C. reshni* Hort. ex Tan  $\times$  *P. trifoliata*) are more lime-tolerant [18, 21, 23, 24].

### 2.3. Flooding

Soil flooding has been widely reported to affect large areas of the world, and generally in relation to poor soil drainage combined with excessive rainfall or irrigation. One major constraint that stems from excess water is the progressive reduction in both the soil  $\text{O}_2$  concentration and redox potential, which leads to the formation of reduced compounds of either chemical or biochemical origin. Accordingly, flooding effects on plants are related mainly to declining aerobic root respiration that impairs ATP synthesis and which, in turn, disrupts plant metabolism and induces a variety of physiological disturbances that alter plant growth [25], including reductions in water flux from roots, hormonal imbalances, altered carbohydrate distribution, deficient nutrient uptake, early leaf senescence and injury in organs, which sometimes precede plant death (Figure 3) [26].

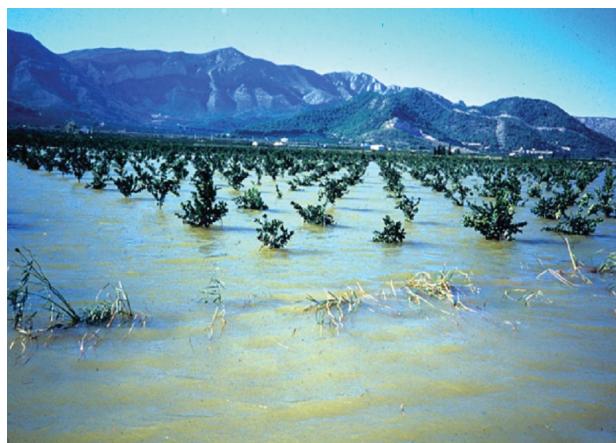


Figure 3. Citrus field affected by flooding stress.

Although the response is variable among species and cultivars, citrus is considered a flooding-sensitive crop and responds to waterlogging by restricting stomatal conductance to prevent water loss [27, 28], likely through hormone-regulation and abscisic acid accumulation in leaves [29]. Under these conditions, net CO<sub>2</sub> assimilation by leaves diminishes [27], which leads to altered carbohydrate distribution [30] and oxidative damage to cells due to excess reactive oxygen species generation [31]. During prolonged soil flooding periods, reduced root hydraulic conductance [28, 32] impairs water uptake, which causes leaf wilting, chlorosis and reduced plant growth [29]. As a result of the root physiology dysfunction, flooding also alters nutrient uptake and, therefore, endogenous concentrations of macro- and microelements can be modified. It has been reported that flooding alters nitrogen (N) pools and their partitioning in citrus as a result of reduced uptake and transport [30]. Moreover, in anaerobic soils, N may be lost through denitrification processes [33] which occur because NO<sub>3</sub><sup>-</sup> is the first electron acceptor to be reduced following O<sub>2</sub> depletion [34]. Additionally, waterlogging also prevents potassium (K) uptake and, therefore, lowers K concentrations in leaves [27, 35], whereas it helps the uptake of other elements by roots, such as copper and manganese [35]. Fe nutrition is seriously damaging as anoxia conditions promote reduced Fe<sup>3+</sup> to Fe<sup>2+</sup> as a result of a lower soil redox potential [25]. Moreover, Fe uptake and plant growth are diminished through the inactivation of the activity of enzymes proton-ATPase and ferric chelate-reductase [36]. Uncontrolled excess Fe<sup>2+</sup> uptake in acidic soils leads to very high Fe concentrations in plant tissues and inhibition of root growth due to free radical generation [37].

#### 2.4. Water deficit

Plants undergo water stress either when the water supply to their roots is limited or the transpiration rate becomes intense, primarily caused by water deficit such as a drought or high soil salinity. Every year, water stress on arable plants in different parts of the world disrupts agriculture. Hence, the ability to withstand such stress is of immense economic importance (**Figure 4**).

Plants attempt to adapt to stress conditions with an array of biochemical and physiological interventions. Rootstocks present genetically determined characteristics that affect plant water relations, which include root system distribution, water and nutrient absorption efficiency, anatomy of vascular elements and carbohydrate availability [38–40]. The ability of rootstocks to supply water and nutrients to plants, through differences in root hydraulic conductance, could be the main factor to influence fruit development in citrus trees as it likely determines the strength of the grafted variety and its tolerance to water stress [41, 42]. A reduction in root hydraulic conductance under water deficit conditions is associated with either substantial anatomical modifications, such as the development of Casparyan bands and suberin lamellae in the exodermis and endodermis [43] or diminished aquaporin activity [28, 44]. Other mechanisms to withstand water stress are higher root-shoot ratios, fewer and smaller leaves, concentrated solutes and carbohydrates, or increased activity of oxidative stress enzymes in leaf cells [45]. As a result of water stress, citrus reduces stomatal conductance (gs), transpiration rate (E) and A<sub>CO<sub>2</sub></sub> [27, 46].



**Figure 4.** Citrus tree seriously affected by water deficit stress.

### 3. Photosynthesis and carbohydrate distribution

Biomass differences between trees are likely related to plants' increased ability to assimilate CO<sub>2</sub> during photosynthesis [47]. Trees on rootstocks that enhance photosynthetic capacity grow more vigorously than on other genotypes [48]. This is reflected on photosynthetic product distribution through the differences induced in scion:stock dry matter ratios, which implies changes in source-sink relationships. A high leaf mass with enhanced  $A_{CO_2}$  results in the translocation of increased C-compounds (mainly sucrose and starch) from shoots to roots, as <sup>13</sup>C labelling experiments have demonstrated [48].

Distribution of photoassimilates comprises a tight competition between vegetative organs and developing fruits. Since the number of flowers produced by citrus species is much larger than the number of fruits harvested, it has been concluded that sucrose supply plays a major role in the regulatory mechanism for citrus fruitlet abscission, mainly at the high intensity time of 'june drop' [49]. This phenomenon is strongly dependent on carbohydrate availability and source-sink imbalances. Consequently, photosynthetic activity appears fundamental to supply the high carbohydrate requirements during fruit set since a drop in  $A_{CO_2}$  results in lower sugar production and increased fruitlet abscission, as reported recently [48] in 'Navel' orange. Moreover, carbohydrate content in leaves, and consequently source:sink imbalance, strongly regulate  $A_{CO_2}$ . Hence, sugar accumulation in citrus leaves is the signal that regulates the feedback mechanism to stimulate photosynthesis [50]. The starch concentration in root bark is related with the sink strength of fruits. A higher starch concentration in the root bark of fruits in phase I (active cell division and slow growth) may induce low fruit set [48]. In phase II, the

starch concentration in root bark tissue lowers as a result of the high carbohydrate demand for fruit growth. Slow fruit growth in phase III leads to new sugar accumulation in roots.

Finally, other factors that may determine photosynthetic differences between genotypes are related to morphological, such as stomatal density, or biochemical facts, such as ribulose-1,5-biphosphate carboxylase activity. The hydraulic conductivity of rootstocks has also been correlated with the CO<sub>2</sub> exchange rates of citrus leaves [39, 51].

#### 4. Rootstock citrus breeding programs

The search for new citrus rootstocks that better perform than those currently used is the major aim of the citrus industry in many countries. New diseases, spread of known diseases and citrus culture under different environmental conditions force the demand for new rootstocks. Attempts have been made in several countries to solve these problems through citrus-rootstock breeding programmes.

Several crosses were made in 1951 using *Poncirus trifoliata* as the male parent at the Citrus Research Centre of the University of California [52]. Some works evaluated many of the hybrids obtained for tolerance to CTV (citrus tristeza virus) [53]. Among the studied hybrids, C-13 (*C. depressa* Hay. × *P. trifoliata*) was rated tolerant. Meanwhile, as far as we know, there have been no reports about the performance of C-13. In addition, the reaction of a hybrid of Shekwasha × Swingle trifoliolate orange to nematode *Tylenchulus semipenetrans* Cobb, indicated low levels of nematode infestation of roots [54]. While screening citrus hybrids for cold hardiness, a hybrid of Shekwasha × trifoliolate orange was reported as having good tolerance levels [55].

In 1974, J.B. Forner began a citrus-rootstock breeding programme using traditional hybridisations at the IVIA (The Valencian Institute of Agrarian Research, Moncada, Valencia, Spain) to obtain new rootstocks tolerant to CTV, salinity and to lime-induced chlorosis and with resistance to *Phytophthora*. To date, more than 500 hybrids have been evaluated to determine their horticultural performance. Of these, four new hybrid rootstocks are now available for better performance in alkaline soils: Forner Alcaide 5 (FA 5) and FA 13 (*C. reshni* Hort. ex Tan × *P. trifoliata*), FA 418 [Troyer citrange (*C. sinensis* × *P. trifoliata*) *C. deliciosa* Ten.] and FA 517 (*C. nobilis* Lour × *P. trifoliata*). These rootstocks have been tested in calcareous soils. The 'Navelina' trees grafted onto FA 5 or FA 13 yielded 40% more than trees on Carrizo citrange, whereas the trees grafted onto FA 5 or FA 13 produced smaller, but similar quality, fruit than those on Carrizo citrange [56, 57].

#### 5. Dwarfing rootstocks

Citrus, like most fruit tree species, are propagated by grafting onto rootstocks that have been selected for their performance under different edaphic conditions or tolerance to diseases. The ability of dwarfing rootstocks to reduce tree vigour has been cited as an important advantage

for fruit-crop growers as it allows management cost savings, and even increased yield per unit area in high-density plantations [58]. Despite its interest, the availability of dwarfing rootstocks in citrus has been scarce until recently, and has been restricted almost exclusively to 'Flying Dragon' (*Poncirus trifoliata* L. Raf var. *monstrosa*). This plant greatly reduces canopy size, increases yield efficiency and produces good fruit quality when used as a rootstock for any citrus cultivar [59]. Despite its resistance to citrus tristeza virus, *Phytophthora* root rot and citrus nematode, it is highly susceptible to iron chlorosis [60], which has likely limited its diffusion in commercial orchards in large citrus cultivated areas.

Fortunately, a few Forner-Alcaide hybrid selections exist which also confer a dwarfing response on scions, in particular FA 517 (Figure 5) and FA 418, whose agronomical behaviour has been tested under field conditions [61–63]. Both rootstocks show lower canopy volumes, but higher yield efficiency when compared with Carrizo citrange, the most extended citrus rootstock in Spain [63]. Moreover, they produce good fruit quality and optimal response when cultured in alkaline soils, one of the main factors that limits crops in Spanish soils [61–63].



**Figure 5.** Influence of Citrus rootstock on tree size response at field conditions. (A) Tree on Forner-Alcaide 517 (dwarfing behaviour) and (B) Forner-Alcaide 5 (normal size).

Growth reduction induced by dwarfing rootstocks has been associated with lower leaf and stem water potentials in the scions grafted onto them compared with those grafted onto vigorous rootstocks, probably due to high hydraulic conductivity resistance, which may cause water deficit in leaves during periods of high evaporative demand and stomata closure [64]. Consequently, dwarfing rootstocks are poorly able to transport water from soil to stems [65]. Although the resistance of bud union to water transport and xylem anatomical characteristics, in particular the number and diameter of vessels, may limit plant growth, carbohydrate distribution is also an important constraint involved in tree response [63, 64]. So, the reduced translocation of photoassimilates from leaves to roots limits root development and also contributes to the greater availability of these compounds in the scion, which results in increased carbon transport towards fruits. This explains the high yield efficiency and good fruit quality that these rootstocks exert on scions.

## 6. Rootstock effects on

### 6.1. Tree vegetative growth

It is widely assumed that rootstocks greatly affect tree size [66–69], which has been noted in some citrus rootstocks (**Table 1**), such as Flying Dragon [64, 70]. This effect is of much interest in citrus breeding works as it cuts yield costs. However, growth evaluations in adult trees are complicated by handling difficulties. Some tree morphology-based parameters allow estimates of plant development under field conditions to be made without destroying plant material. Thus, the trunk cross-sectional area (TCSA) is usually considered to be highly correlated with tree weight and canopy volume [71, 72]. A study carried out in Lane Late orange grafted onto different rootstocks has reported that trees on *C. macrophylla* W. had a smaller TCSA and trunk diameter than Gou Tou Chen (Citrus hybrid of *C. aurantium*) and Cleopatra mandarin trees [73]. Similar TCSA values have been obtained on *C. volkameriana* with 'Clementine' mandarin [66].

Another good parameter to evaluate plant development and relative growth between both tree fractions is the scion/stock ratio. It corresponds to the ratio of the circumference from the scion to that of the rootstock, reflects the difference in the growth rates of each tree fraction, and is used as an indicator of scion/rootstock affinity [74]. The closer this ratio is to a value of one, the better affinity between scion and rootstock is observed, and therefore, less interference in tree growth. In oranges, *C. volkameriana* presents a good scion/rootstock affinity with Lane Late scions (0.94), but lowers to 0.88 with *C. macrophylla* and Cleopatra mandarin [68, 73]. In lemons (*Citrus limon* Burn. F.), the best scion/ratio corresponds to combinations with *C. macrophylla*. Its good agronomical behaviour has allowed these rootstocks to become the most widely used for lemon crops in Spain [67, 75].

Nevertheless, the normal cultural practice in Spain is to form scaffold branches next to the bud union, but this makes TCSA measurements difficult. Under these conditions, canopy volume proves to be a better parameter to evaluate tree size. 'Navelate' trees grafted onto *C. volkameriana* and Cleopatra mandarin presents a larger canopy volume than on C-13 [76]. This tendency has also been observed in 'Navelina' orange trees grafted onto *C. volkameriana* rootstocks [57]. Some new rootstocks obtained in the breeding programme carried out at the IIVIA, in particular rootstocks FA 5 and FA 13, presented an intermediate size [57], while smaller trees were grafted onto FA 418 [77]. In mandarine scions, *C. volkameriana* also conferred the largest size to 'Clausellina' trees compared with Carrizo citrange [78], the most commonly used rootstock in Spain. Once again, mandarin trees on C-13 had the smallest canopy volume and shortest tree height [78].

### 6.2. Yield and yield efficiency

In general terms, citrus trees yield their first crop 2–3 years after planting and these plants reach full production by year 5 or 6. However, Cleopatra mandarin produces a very reduced number of fruits, which is typical of its slow growth tendency in the first few years after

planting, and trees grafted onto this genotype do not reach full production until year 8 [76]. Another exception is FA 418, which is considered to anticipate bear fruit [77].

**Table 1** also lists the yield per tree associated with the main Citrus rootstocks. In lemons, *C. macrophylla* generates high crops in most lemon varieties, while other rootstocks like Carrizo citrange or Cleopatra mandarin induce low yields [67, 75, 79], which is likely linked to their low TCSA [72]. Conversely, good yields were observed in 'Eureka' lemon on Cleopatra mandarin rootstocks [80]. Trees on *C. macrophylla* rootstock produced the highest cumulative yield in 'Lane Late' oranges, and showed no significant differences with trees on Cleopatra mandarin. In contrast, a study into 'Marisol' Clementine, reported that Cleopatra mandarin was the least productive rootstock [68]. However, 'W. Navel' orange trees budded onto Carrizo citrange produced the highest fruit yield, while the lowest corresponded to Cleopatra mandarin [81]. In the other hand, fruit yield of some mandarin trees as 'Fallglo' and 'Sunburst' were not affected by rootstocks [82].

Despite the small tree size with the C-13 genotype, its high yield allows this genotype to present good yield efficiency on a canopy volume basis, followed by Carrizo citrange and *C. volkameriana* [8]. At the far end, the low yield of trees on Cleopatra mandarin means very poor yield efficiency. In 'Lane late' trees, the best yield efficiency corresponded to the trees on *C. macrophylla*, and the lowest yield-efficient trees were those grafted onto Gou Tou, Cleopatra mandarin and FA 418, while *C. volkameriana* offered an intermediate yield efficiency [73, 77]. Similar results have been reported on 'Navelina' orange, who found that the trees on *C. volkameriana* had similar yield efficiency, but lower yield efficiency on Cleopatra mandarin [57]. In line with this, studies in 'Marisol' Clementine and found that all the studied rootstocks achieved similar yield efficiency [68]. These results agree with others on 'Shamouti' orange, 'Nova' mandarin and 'Clementine' mandarin, in Cyprus [66, 83]. Interestingly, the low vigour and high yield efficiency of the trees grafted onto FA 517 and FA 418 indicated that these rootstocks were suitable for high-density plantings to compensate for the reduced productivity of individual trees [63]. Other studies also observed good yield efficiency and low TCSA values in 'Eureka' and 'Lisbon' trees on *C. macrophylla* rootstock [75].

### 6.3. Fruit drop

Fruit drop is a major disorder that comprises fruit yield in citrus orchards. So, growers apply 2,4-DP (2-ethylhexyl ester or dichlorprop-p) to reduce this problem. However, this practice increases cultivation costs. In addition, the excess of production of some cultivars prolongs the harvest period and a significant part of the crop is lost. For these reasons, an excellent trait for rootstocks is to retain ripened fruit. The tendency of fruits to drop increases with plant age, which is strongly regulated by the influence of rootstocks on scions. Thus, C-13 ranks the highest for fruit drop, with values from 51% to 85% when used as a rootstock for 'Navelate' orange, but with values from 40% to 65% when grafted onto Carrizo citrange, Cleopatra mandarin and *C. volkameriana* [76]. In 'Lane Late' orange, Cleopatra mandarin and new hybrids FA 030230, FA 020321, FA 418 and FA 030212 showed low pre-harvest drop, while Carrizo citrange, FA 030127 and FA 13 obtained a fruit drop value above 36% [77].

#### 6.4. Alternate bearing index (ABI)

The ABI analysis, which reflects the difference in yield between two consecutive harvests, shows important new information since it normally differs among rootstocks. Some authors have described that there are some rootstocks which increase the alternate bearing of yields [77], and that this effect is extremely harmful in the commercial varieties that already present this defect. This is the case of the 'Verna' lemon variety, which develops a very alternate behaviour [84]. Some authors have indicated that *C. macrophylla* [73] is one of the rootstocks that presents the most uniform productivity (less than 16% ABI). Good ABI values were also obtained for 'Lane late' orange when grafted onto rootstocks Carrizo citrange, FA 5, FA 030131 and FA 020324 [73]. FA 418 also has a poor alternate effect on Navel orange trees [63]. However, the fruits on Cleopatra mandarin and *C. volkameriana* present a relatively low-moderate ABI values (between 23% and 35%), but exhibit more than 50% alternate bearing on Gou Tou [77]. 'Nova' and 'Clementine' mandarin trees within a wide range of most known rootstocks displayed relatively high ABI values [66]. In contrast, some studies into 'Shamouti' orange and 'Fallglo' and 'Sunburst' mandarin have suggested that alternate bearing is not rootstock-dependent [82, 83].

#### 6.5. Fruit quality variables

In fruits destined to be consumed as fresh fruit, fruit size, juice content and the TSS/TA ratio are most important. Rootstocks have been reported to significantly affect both the external (size, rind thickness, peel colour, etc.) and internal (juice content and colour, pH, total soluble solids, etc.) quality variables of fruit. The influence of several Citrus rootstocks on two of the main quality factors (fruit size and maturity) is listed in **Table 1**.

##### 6.5.1. Fruit size

Rootstocks do not apparently affect fruit size in the first harvest years of citrus crops, but do alter fruit size as the tree age increases. After the ninth harvest, the trees on *C. volkameriana*, C-35 citrange (*C. sinensis* cv. Ruby × *P. trifoliata* cv. Webber Fawcett.) and Carrizo citrange produced the heaviest and largest fruits on mandarin and orange fruits [57, 73, 77, 78]. High values have also been obtained in fruits on some hybrid selections, such as FA 030123 and FA 030142. In contrast, the oranges on Gou Tou and Cleopatra mandarin yielded light and small fruits [73], and the same occurred with the FA 13 hybrid [57]. Forner-Giner et al. [8] have also reported small and light fruits on the trees on Cleopatra mandarin and FA 020326 hybrid selection. In contrast, [68] have reported 'Marisol' clementine and [85] on 'Shamouti' oranges that the trees on sour orange, Carrizo citrange and Swingle citrumelo [*C. paradisi* Macf. × *P. trifoliata*] produced similar fruits in both weight and size terms. Meanwhile, Tuccu et al. [81] have indicated that the fruit weight of 'W. Navel' orange on sour orange was similar to that on Carrizo citrange and Cleopatra mandarin. It is noteworthy that FA 418 has been reported to maintain good fruit growth in orange varieties despite its dwarfing behaviour [63]. In lemons, the trees on *C. macrophylla*, *C. volkameriana* and other less known rootstocks in Spain (*C. sulcata*, *C. taiwanica* Tan. Shim. and *C. ampullacea*) have been found to generate large-sized fruits [72, 79]. In contrast, reduced size in lemon has been observed in the fruits of the Cyprus local variety

'Lapitkiotiki' on Cleopatra mandarin, sour orange and Morton citrange [*C. sinensis* cv. Washington navel × *P. trifoliata* (L.) Raf.] rootstocks [67]. *Citrus amblycarpa* Ochse and Cleopatra mandarin rootstocks have also induced a small fruit diameter in 'Eureka' lemon [79].

#### 6.5.2. Rind thickness

The rind thickness analysis is a good parameter to estimate fruit quality as it is inversely correlated with the amount of juice [77]. Rind thickness extremes 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. This parameter is also influenced by rootstock. Thus, the 'Lane Late' fruits from *C. volkameriana* and *C. macrophylla* have thick rinds, which are thinner when grafted onto Gou Tou and Cleopatra mandarin [73]. Although larger, the fruits of the trees on *C. volkameriana* present thick rinds, while C-13 peels are very thin [8]. In oranges, the FA 418 rootstock also confers thick peel to fruit (>4.8 mm), but this parameter drops to near 4.0 mm in the fruits on Cleopatra mandarin, FA 13, Carrizo citrange and some hybrid selections, such as FA 030212, FA 030127 and FA 030113 [8, 57]. In mandarins, once again *C. volkameriana*, and also Carrizo citrange, present the thickest rind (around 2.3 mm), while the C-13 rootstock induces thin peels, around 2.0 mm [78].

#### 6.5.3. Colour index (CI)

Colour is considered one of the most important external factors of fruit quality as fruit appearance greatly influences consumer acceptance. A coloured fruit on the tree is always ripe, so the risk of selecting immature fruit due to colour is highly improbable, unless they are artificially degreened. A non-destructive method exists that can be applied in the field and in industry to accurately show the apparent degree of fruit maturation in temperate countries [86]. According to [73], oranges with the best external colour are produced on *C. macrophylla* and *C. volkameriana* (CI ~ 1.82), and the worst are yielded on Gou Tou (1.16). Former-Giner et al. [57] also recorded low CI in 'Navelina' oranges on Cleopatra mandarin. In [61], 'Lane late' fruits with the best external colour were produced on Cleopatra mandarin and FA 020324 hybrid rootstocks (CI > 1.30), which showed the most intense orange-coloured skin due to a higher  $a^*$  parameter. However, a high  $L^*$  parameter in the fruits on FA 030131 and 030127 lowered CI to values under 0.45. Carrizo citrange also resulted in attractive orange fruits with a higher CI than 1. Similar values of  $L^*$ ,  $a^*$  and  $b^*$  have been obtained in other studies [87, 73]. The fruit colour index of fruits grafted onto FA 418 was also lower than on other rootstocks [63].

#### 6.5.4. Juice content and colour

In general, the larger the fruit and the thicker the rind are, the lower the juice content is. This applies to the higher juice content of 'Marsh' grapefruits (*Citrus paradise* Macf.) on sour orange than on *C. amblycarpa* or Cleopatra mandarin [88]. In orange, the fruits of *C. volkameriana* and *C. macrophylla* present low juice content [73]. Accordingly, García-Sánchez et al. [10] found that the fruits of 'Clemenules' mandarin on Carrizo citrange had a higher juice percentage and a lower peel percentage than those on Cleopatra mandarin. Contrarily, statistically significant differences in fruit peel thickness and juice content were not found among rootstocks by these

authors: [68] on 'Marisol' clementine, [85] on 'Ortanique' tangor, [81] on 'W. Navel' and [82] in 'Fallgo' and 'Sunburst' mandarins. Forner-Giner et al. [8] also reported a higher juice content in the fruits grafted onto Carrizo citrange, C-13 selection and FA 020326 than on Cleopatra mandarin and *C. volkameriana*. Misra et al. [89] obtained a maximum juice content in the fruits of lemon trees onto trifoliate orange and Cleopatra mandarin, while the lowest content went to *C. taiwanica* [90]. Rootstocks influence juice colour. In [61], the fruits of the trees grafted onto Cleopatra mandarin and Gou Tou were more luminous in colour (a higher  $L^*$  parameter), while those grafted onto *C. macrophylla* and *C. volkameriana* produced fruit with the most intensely orange-coloured skin (higher  $a^*$  parameter). Similar  $L^*$ ,  $a^*$  and  $b^*$  results were obtained on 'Hamlin' and 'Earlygold' by Lee and Castle [87].

#### 6.5.5. Total soluble solids (TSS) and total acid (TA) percentages

The flavour and palatability of citrus fruits vary according to relative levels of TSS, and also to the presence or absence of aromatic or bitter juice constituents [91]. Carrizo citrange, FA 030212 and FA 030230 selections induced higher TSS in the fruits of 'Lane late' navel orange than Cleopatra mandarin, FA 13 and FA 030127 [77], while the lowest values were found when analysing the fruits from the Gou Tou rootstock [73]. Interestingly in clementines, Cleopatra mandarin induced a higher TSS in fruits than in orange varieties, as reported in 'Marisol' and 'Clemenules' studies [68, 92]. It is noteworthy that the high yield recorded by *C. macrophylla*, did not significantly affect its TSS compared with other rootstocks.

In 'Lane late' orange, a low total acid (TA) percentage was found on the fruits of *C. volkameriana*, and with no significant differences with *C. macrophylla*, while the highest acidity was induced by Cleopatra mandarin and Gou Tou [73]. Carrizo citrange, FA 418 and FA 030212 also induced high TA contents, while low levels were induced in the fruits of FA 13 [77]. In contrast, some authors have found that the effects of rootstock on fruit juice acidity were non-significant [10, 81, 93]. Regarding organic-acid content, *C. volkameriana* and *C. macrophylla* induced low total acid values on 'Lane late' orange [73]. The major organic acid in 'Lane late' navel orange was citric acid (0.89–1.15%) and differences were found between genotypes. In contrast, malic acid was not apparently affected by rootstock, with values between 0.29% and 0.31%. These results were also found on calamondin and 'Kozan' orange [94, 95]. Interestingly, high ascorbic acid values have been recorded in the 'Lane late' trees grafted onto Gou Tou and Cleopatra mandarin [73].

The ripeness index (RI), which relates the soluble solid content measured in °Brix and the titratable acidity determined as a percentage of citric acid content in fruit juice, is the most widespread method used to estimate the citrus fruit maturity level. In 'Lane late', the fruits on FA 13 obtained a high RI value, which was low on FA 418, FA 030212 and Carrizo citrange. The fruits of the trees on *C. volkameriana* and *C. macrophylla* also showed high RI values, which were lower in Cleopatra mandarin and *C. volkameriana* [73]. However on 'W. Navel' orange, on 'Rhode Red Valencia' orange and on 'Okitsu' Satsuma mandarin, [81, 93, 96] reported that the effects of rootstocks on the RI were not statistically significant.

#### 6.5.6. Sugar content

Finally, sugars are the major components of citrus juice soluble solids and sweetness of orange juice is intrinsic to its sugar composition. Sucrose is the main sugar present in orange juice, more than 55%, followed by glucose and fructose [73, 95]. The bibliography strongly relates sugar content and rootstock influence on variety. This effect is very important in orange and mandarin fruits [62, 69], and has also been observed in other studies carried out on lemons [75, 79]. Legua et al. [61] also observed high total sugars content in the juice from *C. macrophylla* and Cleopatra mandarin, and low contents from *C. volkameriana*. Similar results have been reported on 'Kozan' and 'Salustiana' oranges [95, 97]. In contrast, some works have found no appreciable significant differences in the juice contents of 'Comune' Clementine, 'Orlando' tangelo and grapefruit on the rootstocks studied therein [98–100].

## 7. Conclusions

Citrus growth is dependent on rootstock effect. Plant responses to abiotic stress conditions where rootstock behaviour plays a key role in tree development. Rootstock influence on the scion's photosynthetic capacity linked to carbohydrate distribution during the annual cycle is a determining factor for plant vegetative and reproductive development. Therefore, rootstock strongly regulates the plant growth, yield and fruit quality of the cultivars. Finally, new dwarfing rootstocks, in which small tree size helps to cut the costs in some cultural practices such as pruning or harvesting, confers very promising and interesting physical and chemical properties to scions which strongly supports their use for citrus production.

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