



## Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418)



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### ARTICLE INFO

#### Article history:

Received 13 March 2014

Received in revised form 11 July 2014

Accepted 24 July 2014

Available online 27 October 2014

#### Keywords:

Citrus

Rootstocks

Dwarfing

C13

Yield

Photosynthesis

### ABSTRACT

The aim of the present work was to study the performance of two new dwarfing citrus rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418) in comparison with Carrizo citrange. To carry out these experiments, rootstocks were budded with Navelina Navel orange scions. In young plants, rootstocks' size-controlling capacity was associated to their hydraulic conductance. In the case of Forner-Alcaide 418, lower hydraulic conductance was related to a smaller lumen area of root xylem vessels with respect to the other rootstocks. Also, hydraulic resistance of bud union segments of the stem was found to decrease with increasing vigour of plants grafted onto the different rootstocks. Furthermore, the graft union may provide resistance to sucrose transport in dwarfing rootstocks, as indicated by the reduced translocation of <sup>13</sup>C-labelled photoassimilates from leaves to roots and reduced soluble sugar and starch concentrations under the graft union. This may result in a deficient carbohydrate supply to roots in dwarfing rootstocks, thereby stunting their growth. Accordingly, small root size and low hydraulic conductivity may cause water deficit in leaves during periods of high evaporative demand, inducing stomatal closure. This leads to a decrease in photosynthetic assimilation rate, which may affect plant growth negatively. These effects occur to a different extent in scions on both dwarfing rootstocks, appearing to be the cause of their reduced growth.

Moreover, plants on both dwarfing rootstocks showed decreased translocation of <sup>13</sup>C-photoassimilates to roots, possibly contributing to higher availability of these compounds in the scion, and resulting in increased <sup>13</sup>C transport towards the fruits. This effect could explain the higher yield efficiencies found in dwarfing rootstocks.

A ten-year long field experiment, with trees grafted onto the three rootstocks, showed that Forner-Alcaide 517 and Forner-Alcaide 418 induced lower canopy volumes and higher yield efficiencies than Carrizo citrange. In addition, our results show both dwarfing rootstocks produce good fruit quality, are suitable for high density plantings and can be cultured in alkaline soils.

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### 1. Introduction

Citrus, like most fruit tree species, are propagated by grafting onto rootstocks selected for their performance in different edaphic conditions or tolerance to diseases. In addition, some rootstocks reduce vegetative growth in scions. These “dwarfing” rootstocks are used to plant high-density orchards, in which the small tree size helps reduce costs in some cultural practices, such as pruning and harvesting.

*Abbreviations:* CC, Carrizo citrange; FA-517, Forner-Alcaide 517; FA-418, Forner-Alcaide 418.

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<http://dx.doi.org/10.1016/j.scienta.2014.07.032>

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Several hypotheses have been proposed to explain rootstock-induced reduction in scion growth. These include nutritional disturbances (Jones, 1976), altered hormone levels (Kamboj et al., 1999; Soumelidou et al., 1994a), partial genetic incompatibility between rootstocks and scions causing necrosis in the graft union (Gur et al., 1968), and vascular malformations in defective graft unions leading to reduced water conductivity (Gur and Blum, 1975). An early anatomical study of the roots of vigorous and dwarfing apple rootstocks indicated that water relations could be involved in dwarfing (Beakbane and Thompson, 1939). This was supported by further works showing that, when climatic transpiration demand is high, leaf and stem water potentials were lower for scions grafted on dwarfing rootstocks than for scions grafted on vigorous rootstocks (Olien and Lakso, 1986; Basile et al., 2003a; Gonçalves et al., 2006). It was suggested that reduced growth induced by dwarfing

rootstocks could be a consequence of their higher hydraulic resistance (Cohen and Naor, 2002; Atkinson et al., 2003; Basile et al., 2003b; Solari et al., 2006b; Nardini et al., 2006). It has also been conjectured that, in some trees, stem or root hydraulic conductance may be related with xylem vessel lumen area (Vercambre et al., 2002; Olmstead et al., 2006; Gonçalves et al., 2007; Tombesi et al., 2010; Rodríguez-Gamir et al., 2010). During periods of high potential evapotranspiration, hydraulic conductance determines xylem capacity to transport water to leaves, resulting in water potential reductions (Tyree and Ewers, 1991). This, in turn, causes stomatal closure to prevent cavitation in xylem vessels and damage to the hydraulic system (Tyree and Sperry, 1988; Jones and Sutherland, 1991; Nardini and Salleo, 2000; Poggi et al., 2007).

Some reports show that, in dwarfing rootstocks, the sites of increased hydraulic resistance may be the root system for peach (Basile et al., 2003b; Solari et al., 2006b) and olive (Nardini et al., 2006) or the graft union for apple (Cohen and Naor, 2002; Cohen et al., 2007; Atkinson et al., (2003)) and cherry (Olmstead et al., 2006). Moreover, in citrus, rootstock hydraulic conductivity has been directly related to vegetative growth capacity of trees (Syvertsen and Graham, 1985; Iwasaki et al., 2011). By contrast, kiwifruit plants grafted onto different clonal rootstocks did not present a direct relationship between tree size and plant hydraulic conductance (Clearwater et al., 2004).

Different mechanisms have been proposed to explain the effect of rootstock induced hydraulic resistance on scion growth. One possibility is its influence on diurnal variations in stem-water potential, which modulates stem elongation (Berman and DeJong, 1997; Basile et al., 2003a). Another possible mechanism is by decreasing gas exchange, since low leaf-specific hydraulic conductivity can limit stomatal conductance (Syvertsen and Graham, 1985; Sperry et al., 1993; Cohen and Naor, 2002; Rodríguez-Gamir et al., 2010), which is regulated by water status (Saliendra et al., 1995; Solari et al., 2006a). This results in reduced net photosynthetic CO<sub>2</sub> assimilation rate, with adverse effects on subsequent shoot growth (Hubbard et al., 2001; Solari et al., 2006a). Cohen and Naor (2002) argued that reduced carbon uptake does not necessarily decrease fruit yield, since the limited carbon resources may be differently distributed in the dwarfed plant, being fruit load the stronger sink. Therefore, despite their low photosynthetic capacity, plants grafted onto dwarfing rootstocks may give good yields with reduced vegetative growth.

The aim of the present work was to provide insight into the relationships between vegetative and reproductive effects of dwarfing rootstocks with hydraulic conductance and carbohydrate allocation. Moreover, Navel orange scions grafted on two new dwarfing rootstocks (FA-517 and FA-418) were tested under field conditions and their behaviour was compared to grafts on Carrizo citrange.

These new rootstocks were obtained in a programme run at the Valencian Institute for Agricultural Research to breed citrus rootstocks by hybridization (Forner et al., 1996). In this programme, more than 500 hybrids were selected for their resistance to citrus “tristeza” virus and were then evaluated in terms of their horticultural performance. Among them, FA-517 (hybrid of *Citrus nobilis* Lour × *Poncirus trifoliata* L. (Raf.)) and FA-418 (hybrid of Troyer citrange × *C. deliciosa* (Ten)) presented dwarfing properties on scions. These were compared with Carrizo citrange (hybrid of *C. sinensis* (L.) Osb. × *P. trifoliata*), which is the main rootstock used in the Mediterranean area and is characterized by inducing high vigour in scions. The study comprised three experiments. In the first, which was performed with 2-year-old plants grown under greenhouse conditions, the hypothesis tested whether the characteristics of xylem vessels from dwarfing citrus rootstocks are determinant of their reduced hydraulic conductance, affecting leaf gas exchange and grafted scion growth. In the second experiment, using five-year-old trees grown in containers, the hypothesis tested whether the graft

union of dwarfing rootstocks limits sugar transport, thereby leading to increased carbohydrate availability above the graft union, which may facilitate fructification. The third experiment was designed to evaluate performance in terms of growth and yield parameters in Navel orange trees grafted onto the three rootstocks, under field conditions, over a ten-year period.

## 2. Material and methods

### 2.1. Experiment I

The aim of this experiment was to examine possible relationships between the vegetative growth of Navel orange plants grafted onto CC, FA-517 and FA-418 rootstocks with xylem anatomical characteristics, hydraulic conductance and diurnal variations in gas exchange parameters.

Carrizo citrange, FA-517 and FA-418 seeds were germinated in a glasshouse using a sterile substrate composed of peat, coconut fiber, sand and perlite (50:25:20:5) supplemented with 1.38 g kg<sup>-1</sup> calcium superphosphate and irrigated twice weekly for 16 weeks with the following nutrient solution: 1.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 1.5 mM KNO<sub>3</sub>, 1 mM MgSO<sub>4</sub>, 1.2 mM H<sub>3</sub>PO<sub>4</sub>, 17.9 μM Fe-EDDHA, 23.2 μM H<sub>3</sub>BO<sub>3</sub>, 27.2 μM MnSO<sub>4</sub>·H<sub>2</sub>O, 3.8 μM ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.27 μM MoO<sub>3</sub> and 0.25 μM CuSO<sub>4</sub>·5H<sub>2</sub>O.

Then, seedlings were planted individually in opaque plastic pots of 10 l filled with a steam sterilized substrate composed of siliceous sand (40%), peat moss (60%), calcium superphosphate (1.5 kg m<sup>-3</sup>), dolomite (2.22 kg m<sup>-3</sup>) and CaCO<sub>3</sub> (3 kg m<sup>-3</sup>). After six months, seedlings of each rootstocks were selected for uniformity of size and half of them were budded with Navelina Navel orange scions with T budding procedure in summer.

Rootstock seedlings and then grafted plants were grown under glasshouse conditions with supplementary light (50 mmol m<sup>-2</sup> s<sup>-1</sup>, 400–700 nm) to extend the photoperiod to 16 h. Temperatures ranged between 16–18 °C at night and 26–28 °C by day. Relative humidity was maintained at approximately 80%. Pots were irrigated twice weekly with the following nutrient solution: 3 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 3 mM KNO<sub>3</sub>, 2 mM MgSO<sub>4</sub>, 2.3 mM H<sub>3</sub>PO<sub>4</sub>, 17.9 μM Fe-EDDHA, 23.2 μM H<sub>3</sub>BO<sub>3</sub>, 27.2 μM MnSO<sub>4</sub>·H<sub>2</sub>O, 3.8 μM ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.27 μM MoO<sub>3</sub> and 0.25 μM CuSO<sub>4</sub>·5H<sub>2</sub>O.

Nutrient solution pH was adjusted to 6.0 with 1 M KOH or 1 M H<sub>2</sub>SO<sub>4</sub>. Two litres of solution per pot were used in each watering event. Excess solution drained out of the pot, thereby avoiding salt accumulation in the sand.

Six seedlings and six grafted plants of each rootstock were randomized over the experimental area and a row of plants, not included in the experiment, was placed around the perimeter as a buffer row.

Two years after seeds were germinated (fourteen months after grafting for young trees), the above plants were harvested and divided into leaves, shoots, coarse roots and fibrous roots (less than 2 mm Ø), which were washed and weighed. Total leaf area was determined by measuring individually all collected leaves per plant. Leaf area determinations were performed with a Li-Cor Li-3100 area metre (Li-Cor, inc. Lincoln, Nebraska, USA). Additionally, trunk diameters, 7 cm above and below the budding union, were measured to calculate the cross-section area. Organs were then dried in a forced-draft oven at 65 °C for 48 h and reweighed. During a summer day of the second year, diurnal variations in stomatal conductance (g<sub>s</sub>), transpiration (E), and net CO<sub>2</sub> assimilation (A<sub>CO<sub>2</sub></sub>) were recorded. Also, independent sets of six grafted plants on each rootstock were used to determine the hydraulic conductivity of organs

### 2.2. Experiment II

The aim of this second experiment was to determine the influence of the studied rootstocks on gas exchange parameters and

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