

## Production efficiency and quality in seedlings of the 'Pera' orange interstocked with nucellar plantlet tissue<sup>1</sup>

Eficiência de produção e qualidade de mudas de laranjeira 'Pêra' interenxertadas por tecidos de plântulas nucelares

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**ABSTRACT** - Production of interstocked seedlings of the 'Pera' orange can be achieved through inarching of a rootstock on nucellar seedlings, with the subsequent grafting of a new scion. By this process, the interstocked seedling can be obtained in less time, but production efficiency is low, and no data on quality standards are available. The aim of this study was to evaluate the quality, time and production efficiency of 'Pera' orange (PO) seedlings interstocked with two types of nucellar plantlet tissue, using the 'Flying Dragon' trifoliolate as rootstock. The experimental design was of randomised blocks, with four treatments, six replications and ten plants per plot. For the technique employed, nucellar plantlets of the 'Seleta' orange (SO) or Rangpur Lime (RL) were selected, followed by inarching with 'Flying Dragon' rootstock (FD) and budding with scions of the 'Pera' orange. The treatments consisted of different interstock combinations, PO/SO/FD and PO/RL/FD, and of non-interstock seedling combinations, PO/RL and SO/FD. Seedlings of the 'Pera' orange produced with interstocks of nucellar plantlets of the Rangpur Lime give seedling architecture of better quality. The interstocked seedlings were obtained 17 months after sowing the FD, with standards of physical quality similar to those required for commercial production, and with a production efficiency greater than 80%. When grown alone, FD requires more time to reach a sufficient diameter for grafting, delaying the production of seedlings.

**Key words:** Planting density. *Citrus sinensis* (L.) Osbeck. *Poncirus trifoliata* var. Monstrosa. Overcoming incompatibility.

**RESUMO** - A produção de mudas de laranjeira 'Pêra' interenxertadas pode ser realizada através da subenxertia de um porta-enxerto em mudas nucelares, com posterior enxertia de uma nova copa. Por esse processo, a muda interenxertada pode ser obtida em menor tempo, mas sua eficiência de produção é baixa e não se dispõem de dados sobre seu padrão de qualidade. Objetivou-se, com esse trabalho, avaliar a qualidade, o tempo e a eficiência de produção de mudas de laranjeira 'Pêra' (LP) interenxertadas por dois tipos de tecidos de plântulas nucelares, tendo o trifoliolado 'Flying Dragon' como porta-enxerto. O delineamento experimental utilizado foi em DBC, com quatro tratamentos, seis repetições e dez plantas por parcela. Na técnica utilizada, plântulas nucelares da laranjeira 'Seleta' (LS) ou do limoeiro 'Cravo' (LC) foram selecionadas e, posteriormente, subenxertadas com o porta-enxerto 'Flying Dragon' (FD) e enxertadas com a copa de laranjeira 'Pêra'. Os tratamentos foram constituídos por diferentes interenxertos, nas combinações LP/LS/FD e LP/LC/FD e por mudas sem interenxertos, nas combinações LP/LC e LS/FD. Mudas de laranjeira 'Pêra' produzidas com interenxertos de plântulas nucelares do limoeiro 'Cravo' proporcionam melhor qualidade na arquitetura da muda. As mudas interenxertadas foram obtidas aos 17 meses após semeadura do FD, com padrões físicos de qualidade semelhantes aos exigidos para produção comercial e com eficiência de produção superior a 80%. O FD quando cultivado sozinho demanda maior tempo para atingir diâmetro suficiente para enxertia, atrasando a produção das mudas.

**Palavras-chave:** Adensamento de plantio. *Citrus sinensis* (L.) Osbeck. *Poncirus trifoliata* var. monstrosa. Superação de incompatibilidade.

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## INTRODUCTION

Brazil is the largest producer of oranges in the world, with a production of 16.2 million tons in a harvested area of 688.248 ha, and an approximate yield of 23.5 tons ha<sup>-1</sup> in 2015. Average productivity for the state of São Paulo, the largest domestic producer, was approximately 30 tons ha<sup>-1</sup>. In the other states, productivity was below the national average, and in Rio de Janeiro, productivity was 17.9 tons ha<sup>-1</sup> for the same year (FAO, 2016, IBGE, 2016). The increase in citrus productivity has been made possible by higher planting densities and the use of rootstocks that reduce plant size, such as trifoliolate trees, which have been evaluated for some cultivars and under different conditions of soil and climate (CANTUARIAS-AVILÉS *et al.*, 2011; LIMA *et al.*, 2014; MADEMBASSY; LEMERRE-DESPREZ; LEBEGIN, 2012; PORTELLA *et al.*, 2016; TEÓFILO SOBRINHO; POMPEU JUNIOR; FIGUEIREDO, 2012).

The ‘Hamlin’, ‘Pera’, ‘Valencia’ and ‘Natal’ oranges are the most planted in São Paulo to meet the needs of industry (CITRUS BR, 2010). Among these cultivars, the ‘Pera’ orange has high acceptance in both the fresh-fruit industry and the internal market, with its cultivation being more widespread in the country (PIO *et al.*, 2005, SALIBE, TEÓFILO-SOBRINHO, MÜLLER, 2002). For this cultivar, the availability of rootstocks is more restricted, since grafting incompatibility exists between this scion and several of the rootstocks used, among them the trifoliate and their hybrids (POMPEU JUNIOR; BLUMER, 2014).

The incompatibility of the ‘Pera’ orange with trifoliate and other rootstocks, such as the ‘Volkamer’ lemon, is well documented (GIRARDI; MOURÃO FILHO, 2006; POMPEU JUNIOR; BLUMER, 2014) and the interstocking of another genotype between the scion and rootstock is needed to overcome this type of incompatibility (GIRARDI; MOURÃO FILHO, 2006; GUILHERME *et al.*, 2014).

Interstocking consists of the introduction of tissue of a different genotype (filter), compatible with the scion and rootstock. The interstocked plant presents two grafting regions, and is composed of three genetically different parts, corresponding to the scion/interstock/rootstock (HARTMANN *et al.*, 2011). Girardi and Mourão Filho (2006) evaluated the yield of interstocked seedlings of the ‘Pera’ orange by means of two consecutive buddings, using the ‘Valência’ or ‘Hamlin’ oranges or the ‘Sunki’ or ‘Cleopatra’ tangerines as interstocks, and the ‘Swingle’ citrumelo and ‘Volkamer’ lemon as rootstocks, thereby obtaining seedling formation at 17 months after sowing the rootstocks.

Guilherme *et al.* (2014) obtained interstocked seedlings of the ‘Pera’ orange using inarching in the production process. In the above work, nucellar seedlings of the Rangpur Lime were inarched with ‘Flying Dragon’ rootstock, and later budding with ‘Pera’ orange as scion, which allowed for anticipation of grafting. With this process, the interstocked seedling can be obtained in less time, but its production efficiency is still low, notably due to the low rate of graft success, and no data on quality standards being available for these seedlings. The aim of this work therefore, was to evaluate the quality, efficiency and production time of seedlings of the ‘Pera’ orange interstocked with nucellar plantlet tissue, using the ‘Flying Dragon’ trifoliolate as rootstock.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse protected by plastic cover and anti-aphid screen, at the Darcy Ribeiro North Fluminense State University (UENF), Campos dos Goytacazes, in the north of the State of Rio de Janeiro (RJ). The production period for the seedlings was from June 2014 to March 2016. The design was of randomised blocks, with four treatments, six replications and 10 seedlings per plot. The treatments under evaluation are described in Table 1.

Seeds of the Rangpur Lime (RL) were obtained from Embrapa Santa Maria, in the State of Rio Grande do Sul. Seeds of the trifoliolate ‘Flying Dragon’ (FD) and the ‘Seleta’ orange (SO), were extracted from the mature fruit of mother plants located in the experimental area of UENF, Campos dos Goytacazes, RJ.

The mother plants were around five years of age, in good vegetative state, with regular fruit production and with no symptoms of disease. Before planting, seeds of the RL, SO and FD were chemically treated by immersion in 1 L of aqueous solution containing sodium hydroxide (10 g L<sup>-1</sup>), sodium hypochlorite (150 ml L<sup>-1</sup>) and 12 N hydrochloric acid (2 ml L<sup>-1</sup>), for 45 minutes, and shaken every 15 minutes to remove the tegument, as per the methodology proposed by Altoé *et al.* (2008).

The RL and SO were sown in tubes of 280 cm<sup>3</sup>. The FD was sown thirty days after planting the RL and SO, in tubes of 50 cm<sup>3</sup>. The tubes were filled with Basaplant® Vegetable BX commercial substrate. Added to the substrate were 3.0 g L<sup>-1</sup> Osmocote® slow release fertiliser (14-14-14), 5.0 g L<sup>-1</sup> single superphosphate, and 13.0 g L<sup>-1</sup> limestone. Two seeds were allocated per tube, with five times more seed being sown than the number of seedlings needed for the experiment, which allowed atypical seedlings to be eliminated in the thinning carried out 60 days after sowing.

**Table 1** - Description of the treatments used to obtain interstocked seedlings of the 'Pera' orange (PO), and to obtain controls by direct grafting onto rootstocks of the Rangpur Lime' (RL) or 'Flying Dragon' (FD)

Treatment	Scion	Interstock	Rootstock	Grafting method
1	PO	SO	FD	Budding <sup>(2)</sup> +Inarching <sup>(3)</sup>
2	PO	RL	FD	Budding <sup>(2)</sup> +Inarching <sup>(3)</sup>
3	PO	-----	RL	Budding <sup>(1)</sup>
4	SO	-----	FD	Budding <sup>(1)</sup>

<sup>(1)</sup>on the rootstock; <sup>(2)</sup> on the interstock; <sup>(3)</sup> below the bark of the interstock. SO = 'Seleta' orange

At 120 days after sowing, the RL and SO seedlings reached an average height of 15 cm, and were transplanted to 7.0 L trapezoidal pots, filled with Basaplant® Hortaliças BX commercial substrate. To the substrate were added 3.0 g L<sup>-1</sup> Osmocote® slow release fertiliser (17-07-12), 5.0 g L<sup>-1</sup> single superphosphate, and 13.0 g L<sup>-1</sup> limestone.

The techniques used to produce the interstocked seedlings were adapted from Guilherme *et al.* (2014), with modifications referring to the use of sweet orange as the interstock, and to the cutting time of the interstock root system. The process is described below. When the FD seedlings reached a height of 20 cm, 150 days after sowing, they were transplanted to the trapezoidal pots where the SO or RL seedlings were already being cultivated. A small hole (50 cm<sup>3</sup> in volume) was opened adjacent to each SO or RL seedling to transplant the FD. At 60 days after transplanting, the FD was inarched under the stem bark of the SO or RL seedlings by an inverted 'T' cut, in which the apex of the FD was inserted, cut into a single bevel at a height of 15 cm from the collar. The inarch grafts were protected with parafilm. The seedlings were supported using bamboo stems, being an alternative method for the production of interstocked seedlings.

At 270 days after sowing, the SO or RL seedlings grown to form the interstocks, reached a stem diameter of between 6 and 8 mm, measured 10 cm above the line of inarching, and were grafted with shoots of the 'Pera' orange (30 days after inarching), comprising treatments 1 and 2.

At 90 days after inarching the FD, a girdle, 3 cm in height, was cut 5 cm below the line of inarching in the stem of the SO or RL, and the central region of the girdle strangulated with smooth, flexible wire. The final separation of the root system of the SO and RL interstocks from the shoots was carried out 180 days after inarching with FD, by means of a transversal cut made 1 cm below the line of inarching, leaving the seedling with the root system of the FD rootstock only.

At 270 days after sowing, seedlings of the RL rootstock, which comprised treatment 3, reached

diameters of between 6 and 8 mm, measured 15 cm from the collar, and were grafted with shoots of the 'Pera' orange. Seedlings of FD rootstock that made up treatment 4, reached diameters of between 6 and 8 mm at 470 days and were grafted with the 'Seleta' orange. Treatments 3 and 4 consisted of the controls, corresponding to the conventional method of seedling production with no interstocks.

In all treatments, sprouting of the grafted shoots was stimulated by cutting the aerial part of the rootstock 10 cm above the graft, 25 days after grafting. After the shoots had sprouted, they were grown until reaching a height of 50 cm, when a second cut of the aerial part of the rootstock was made.

During the experiment, cover fertilisation was carried out periodically with a solution of 5 g L<sup>-1</sup> potassium nitrate and 2 g L<sup>-1</sup> calcium nitrate, with 10 ml of each solution being applied per pot. The leaves were sprayed with a solution of 1 g L<sup>-1</sup> copper oxychloride, 1 g L<sup>-1</sup> boric acid, 4 g L<sup>-1</sup> magnesium sulphate, 3.5 g L<sup>-1</sup> zinc sulphate, 2.5 g L<sup>-1</sup> of manganese sulphate and 2.5 g L<sup>-1</sup> urea, as per the methodology used by Serrano *et al.* (2006).

Irrigation was carried out daily from sowing to transplanting, using a fine screen watering can. After transplanting, the water was applied directly to the substrate with the aid of a hose, to avoid wetting the shoots. Air temperature and humidity inside the greenhouse were monitored with the model 3030.15 Climate Data Logger digital weather station. The minimum and maximum temperatures recorded during the experiment were 14.7 and 44.8 °C respectively, while the minimum relative humidity was 44.8% and the maximum was 100%.

The following characteristics were evaluated:

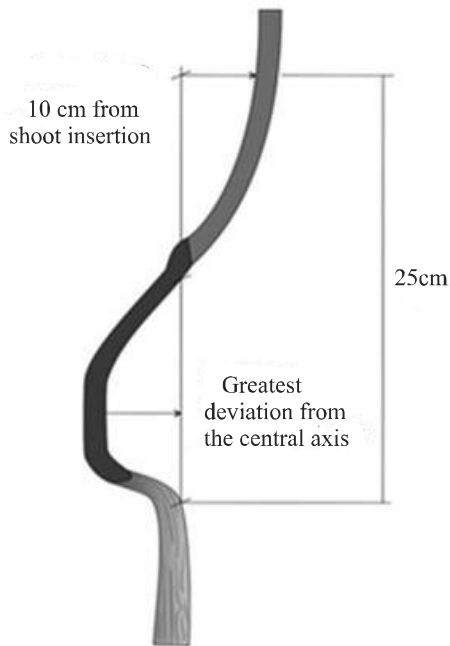
- Percentage survival of the shoots and inarch grafts. Living shoots were considered those that showed green colouration and those with sprouting; graft success for the inarching was evaluated from the union or necrosis of the inarch grafts.

- Stem diameter of the rootstocks, interstocks and inarch grafts, 5 cm from the graft line (monthly), and

seedling height after grafting the shoots, measured from the collar.

Measurements were taken of the deviation, in degrees, of the distance of the graft and interstock relative to the central axis of the seedling. These distances were measured using a millimetre rule, and the angles found by means of trigonometric relationships. For the seedlings with no interstock, this distance was measured 10 cm above the graft line. In the interstocked seedlings, two measurements of the deviation were taken. The first was determined relative to the distance of the graft of the orange scion in relation to the main axis, measured 10 cm above the graft line. The second was determined relative to the distance of the interstock from the main axis (Figure 1).

**Figure 1** - Illustrative diagram of the stem of an interstocked seedling, characterising the regions for taking the measurements to obtain the degree of deviation of the graft and interstock in relation to the central axis of the seedling



Leaf area (LA), shoot dry weight (SDW), root volume (RV) and root dry weight (RDW) were evaluated only at the end of the seedling-production phase. For this, five seedlings from each treatment were cut close to the collar. The leaf area was evaluated with the Licor model LI-3100 meter, Lincoln, NE, USA. Root volume was determined by placing the roots in a graduated beaker containing a known volume of water; the root volume was obtained by water displacement. To obtain the dry weight, seedling parts (leaves, stems and roots)

were separated and packed, placed to dry in a forced air circulation oven at 70 °C for 72 hours, and then weighed on a precision scale.

The results were submitted to analysis of variance and the means compared by Tukey's test at 5% significance. For the characteristics of height and diameter, where evaluations were made over time, the data were analysed in plots subdivided in time, and the means submitted to regression analysis.

The values for percentage survival and graft success were transformed by arcsine  $\sqrt{(X + 0.5)}$ , where  $x$  is the observed value of the variable as a percentage.

## RESULTS AND DISCUSSION

There was no difference between shoot survival for the seedlings with or with no interstocks, showing that the filter used did not interfere with this result (Table 2).

There was no effect from the interaction between the factors combination and period of evaluation. For all treatments, there was a reduction in percentage graft success in the days after grafting. This effect was linear, and can be represented by equation 1:

$$\hat{Y} = 98.56 - 0.0593X \quad (r^2 = 0.83^*) \quad (1)$$

where:  $\hat{Y}$  corresponds to the percentage graft success and  $X$  to the number of days after grafting.

There was a trend for the interstocked seedlings to present a greater reduction in graft success over time.

It should be noted that from 120 to 150 days the root system of the interstocks was cut, and the shoots from the PO/SO/FD and PO/RL/FD combinations were nourished by the root system of the 'Flying Dragon' rootstock only. According to Hartmann *et al.* (2011), the water in interstocked seedlings needs to traverse two successive barriers, created by the two grafting regions, which could result in the loss of turgor in the shoots, and in the results seen in the present work.

Although the rate of graft success for the shoots reduced over time, the results were higher than found by Guilherme *et al.* (2014) for interstocked seedlings, who obtained averages of 25% graft success in plants of the 'Pera' orange interstocked with the Rangpur Lime and inarched with FD, and of only 3.6% when the inarch graft was the 'Swingle' citrumelo. The above authors girdled the interstock stems 35 days after grafting, while in the present work the girdle was cut at 60 days.

In the work of Guilherme *et al.* (2014), the root systems of the interstocks were cut 60 days after grafting

**Table 2** - Mean values for percentage graft success in shoots of the 'Pera' (PO) or 'Seleta' (SO) orange, grafted onto different combinations of rootstocks and interstocks

Combination n.s*	Days after grafting				
	30	60	90	120	150
PO/SO/FD	95.0	91.7	86.7	86.7	85.0
PO/RL/FD	96.7	91.7	90.0	90.0	88.3
PO/RL	98.3	98.3	98.3	98.3	98.3
SO/FD*	100	98.3	96.7	96.7	96.7
Mean	98.3	93.9	91.7	91.7	90.5 s**
CV%(A)	2.07				
CV%(B)	1.07				

n.s\*= non-significant and s\*\*= significant differences by F-test at 5% probability. Grafting of the 'Seleta' orange onto the 'Flying Dragon' (FD) was done 480 days after sowing, and graft success was evaluated from 510 to 630 days after sowing the rootstock. RL = Rangpur Lime

the shoots of the 'Pera' orange, whereas in the present work this cut was made 150 days after grafting. This extra time may have promoted better healing of the tissue, with the formation of a new xylem and phloem, establishing the vascular connections between rootstock and interstock, and may have contributed to the maintenance of turgor in the shoots and consequently, to greater percentage graft success.

Graft success in the inarch grafts did not differ between treatments (Table 3). These percentages showed a reduction over time for all treatments. This effect was linear, and can be represented by equation 2:

$$\hat{Y} = 99.8 - 0.0281X \quad (r^2 = 0,71^*) \quad (2)$$

where:  $\hat{Y}$  corresponds to the percentage graft success of the inarch graft and  $X$  corresponds to the number of days after inarch grafting.

Thirty days after inarching, 100% survival of the inarch grafts was seen. It should be noted that before 180 days the seedlings had two root systems. During this

period the root system of the interstocks (SO and RL) was cut, with the seedlings being nourished only by the root system of the FD. Thus, in cases where the vascular connections between inarch graft and interstock were not established, the shoots of the 'Pera' orange died.

Percentage graft success for the inarch grafts evaluated 180 days after inarching for the PO/SO/FD and PO/RL/FD combinations were 96.7 and 91.7%, respectively (Table 3). Guilherme *et al.* (2014), evaluating production in interstocked seedlings of the 'Pera' orange, obtained 25 and 18.7% survival for 'Pera' orange shoots interstocked onto Rangpur Lime or 'Bahia' Orange onto 'Flying Dragon' rootstock, respectively.

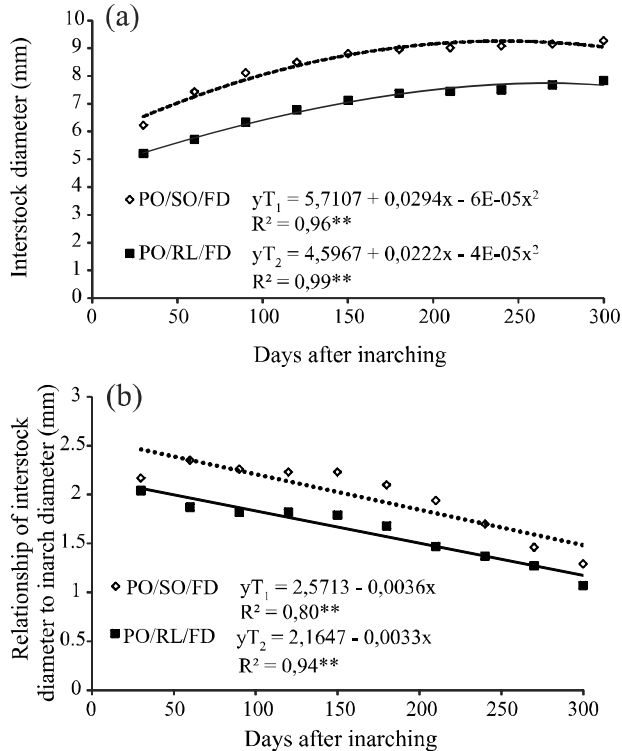
The mean interstock diameter for the PO/SO/FD combination was higher than that of the PO/RL/FD combination, with values of 9.3 and 7.8 mm respectively at 300 days after inarching (Figure 2a). Girardi and Mourão Filho (2006), evaluating the production of 'Pera' orange, using interstocks of the 'Pera', 'Valencia' or 'Hamlin' orange, or the 'Sunki' and 'Cleopatra' tangerines, found

**Table 3** - Mean values for inarch percentage graft success in *Poncirus trifoliata* var. *monstrosa* 'Flying Dragon' (FD) below the stem of *Citrus limoni* Rangpur Lime or *Citrus sinensis* 'Seleta', from 30 to 180 days after inarching

Combination n.s*	Days after inarching					
	30	60	90	120	150	180
PO/SO/FD	100	98.3	98.3	98.3	98.3	96.7
PO/RL/FD	100	95.0	95.0	95.0	95.0	91.7
Mean	100	96.7	96.7	96.7	96.7	94.1 s*
CV% (A)	0.53					
CV% (B)	0.68					

n.s\*= non-significant and s\*\*= significant differences by F-test at 5% probability. SO = 'Seleta' orange; PO = 'Pera' orange; FD = 'Flying Dragon' and RL = Rangpur Lime

**Figure 2** - Diameters of the ‘Seleta’ orange (SO) and Rangpur Lime (RL) interstocks, measured 5 cm above the line of inarching, from 30 to 300 days after inarching (a), and differences between the stem diameter of the interstocks relative to the Flying Dragon rootstock (FD) from 30 to 300 days after inarching (b). \*significant at 5% probability by F-test



higher values than those of this work, with averages of 9.0 and 9.5 mm at 100 days after the interstocks were grafted onto the ‘Swingle’ and ‘Volkamer’ rootstocks respectively.

On the other hand, different results from this work were found by Guilherme *et al.* (2014) when evaluating the Rangpur Lime and ‘Bahia’ orange as interstocks between the ‘Pera’ orange and the FD rootstock, with mean diameters of 8.2 and 3.5 mm respectively at 150 days after grafting. Although the SO interstock presented higher values for stem diameter, the greatest similarity between the interstock and rootstock diameters was seen with the combination PO/RL/FD, with a difference of 1.07 mm, while with the combination PO/SO/FD there was a difference of 1.29 mm (Figure 2b). This result indicates that RL as interstock can provide seedling architecture of better quality.

The PO/RL combination displayed the largest increment in rootstock stem diameter for all the periods under evaluation, reaching a mean stem diameter of 10.8 mm for RL at 18 months after sowing. In all

combinations, FD had a similar mean value for diameter, of 7.3, 7.3 and 7.2 mm respectively for the PO/SO/FD, PO/RL/FD and SO/FD combinations at 17 months after sowing (Figure 3). However, it was found that the stem diameter of FD in the SO/FD combination, reached a mean diameter greater than in the PO/SO/FD and PO/RL/FD combinations in the earliest evaluations, but was equal to the values obtained with the interstocked seedlings 240 days after inarching.

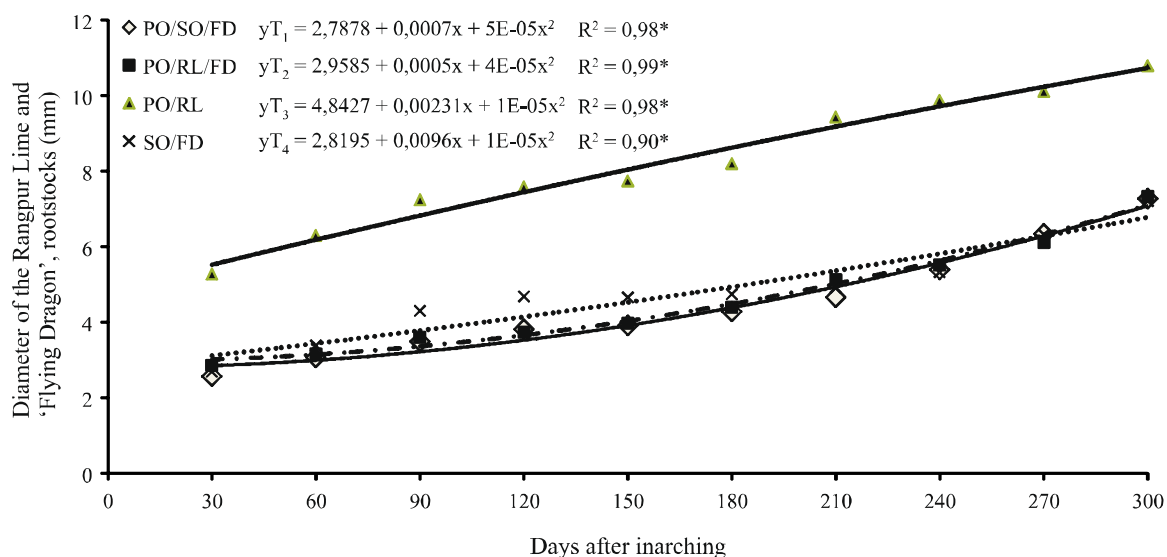
In the earliest evaluations, the smaller increase in the diameter of the inarch-grafted FD may have occurred due to competition between the two root systems for photoassimilates, agreeing with the results of Setin, Carvalho and Matos Júnior (2009), who reported that plants of the ‘Valencia’ orange inarch-grafted with double and quadruple rootstocks showed a smaller increase in stem diameter, the authors relating this fact to the probable competition of the inarch grafts for photoassimilates. Girardi and Mourão Filho (2006) found higher values for stem diameter than those obtained in this study when evaluating the production of ‘Pera’ orange seedlings interstocked onto rootstocks of the ‘Swingle’ citrumelo and ‘Volkamer’ lemon, with mean values of 14, 6 and 12.4 mm respectively 17 months after sowing the rootstock.

The results obtained in this work differ from those obtained by Girardi and Mourão Filho (2006), probably due to the lower vigour presented by the ‘Flying Dragon’ rootstock in relation to the other rootstocks. Setin, Carvalho and Matos Júnior (2009) suggest that the smaller stem diameter seen in the ‘Valencia’ orange inarch-grafted with double and quadruple rootstocks may be related to a probable disadvantage in the competition for photoassimilates between the root systems that constitute more sinks for the same plant. However, scions that are more vigorous may direct more reserves to the root systems of less vigorous rootstock, as is the case of the FD.

Seedling height in the ‘Pera’ orange seedlings for the PO/RL combination (with no interstock) was greater than seedling height in the other treatments. The interstocked seedlings (PO/SO/FD and PO/RL/FD) had similar heights to each other, and similar to the seedlings of the SO/FD combination (with no interstock), but for different production times (Table 4).

The time needed to reach the minimum height required for marketing was 15, 17 and 21 months for the PO/RL, PO/SO/FD and PO/RL/FD, and SO/FD combinations respectively. The seedlings with FD as the rootstock, with or without an interstock, required more time to reach commercial height.

**Figure 3** - Stem diameter of the Rangpur Lime (LR) and trifoliolate 'Flying Dragon' (FD) rootstocks, and the Rangpur Lime and 'Seleta' orange interstocks, for different scion combinations, from 30 to 300 days after inarching. \*significant at 5% probability by F-test. PO = 'Pera' orange



**Table 4** - Mean values for leaf area, shoot dry weight, root volume, root dry weight and height in seedlings of the 'Pera' (PO) and 'Seleta' (SO) oranges grafted onto the Rangpur Lime (RL) or 'Flying Dragon' (FD)

Combination	Leaf area (cm <sup>2</sup> )	Shoot dry weight (g)	Root volume (mm <sup>3</sup> )	Root dry weight (g)	Seedling height (cm)
PO/SO/FD	1685.13 b	30.53 b	49.20 b	10.62 b	105.35 a
PO/RL/FD	1944.17 b	29.47 b	45.00 b c	10.24 b	68.88 b
PO/RL	3192.40 a	52.33 a	84.40 a	21.82 a	67.23 b
SO/FD	494.20 c	10.68 c	33.80 c	6.94 b	59.92 b
CV%	16.91	12.85	15.77	19.31	10.68

Mean values followed by the same letter do not differ between the evaluated combinations by Tukey's test at 5% probability

This result is corroborated by those obtained by Rodrigues *et al.* (2016), who report that seedlings produced on the FD rootstock need a longer period of formation, due to the lower vigour of this genotype.

However, it could be seen in the present study that inarching the FD, in already constituted scions, reduced the formation time of the interstocked seedlings in relation to the seedlings grafted directly onto the FD, as was the case of the 'Seleta' orange.

Girardi and Mourão Filho (2006) obtained greater values for height in grafts of the 'Pera' orange than those found in this study, using interstocks of the 'Pera', 'Valencia' and 'Hamlin' oranges and the 'Sunki' and 'Cleopatra' tangerines on 'Swingle' citrumelo and 'Volkamer' lemon rootstock. The shorter length of the grafts, and lesser growth of the interstocked seedlings seen in this study may be due to the lower vigour of

the FD rootstock. Alcântara *et al.* (2013) reported that the lesser development of *Citrus sinensis* 'Valencia' seedlings grafted onto FD rootstock, resulted from the higher hydraulic resistance presented by this rootstock in relation to the more vigorous rootstocks.

Mean values for leaf area (LA) and shoot dry weight (SDW) (leaves + stem) differed between treatments. Greater values for LA and SDW were found in seedlings obtained with the PO/RL combination with no interstock, in relation to the interstocked seedlings. Among the interstocked seedlings, the values were equal (Table 4). The smallest values for LA and SDW were found with the SO/FD combination; it should be noted however that with this treatment, the LA of the 'Seleta' orange was measured at a different time than the 'Pera' orange, since the FD rootstock did not reach the necessary diameter for grafting at the same time as the other treatments.

Girardi and Mourão Filho (2006) obtained a greater value for SDW in the 'Pera' orange to those found in this work, for seedlings interstocked with tissue of the 'Pera', 'Valencia' and 'Hamlin' oranges, and 'Sunki' and 'Cleopatra' tangerines, at 17 months after sowing the 'Swingle' and 'Volkamer' lemon rootstocks. Whereas, Alcântara *et al.* (2013) obtained values for LA in 'Valencia' orange seedlings grafted onto FD rootstock close to those found in this study, with mean values of 2,200 cm<sup>2</sup> at 26 months, noting that this result was obtained without the use of an interstock.

There was a difference between the mean values for root volume (RV) and root dry weight (RDW) in the seedlings from the four treatments (Table 4). The root system of the seedlings produced with the PO/RL combination had a mean value greater than the other combinations, both in volume and root dry weight. Among the seedlings that had FD as rootstock, those of the PO/SO/FD combination had a higher value for RV in relation to the seedling with no SO/FD interstock and the PO/SO/FD interstocked seedlings. The greater accumulation of RDW in the RL rootstock was expected, since it has a root system that is more branched, with more pilous structures, as reported by Wutscher (1998), while the trifoliate FD has a less vigorous root system.

However, for the PO/SO/FD and PO/RL/FD combinations, an increase of 28.2 and 33.5% respectively was seen in mean root volume in relation to the combination SO/FD. These results suggest that inarching had a positive effect on the growth of the FD root system. On the other hand, the differences between the interstocked seedlings and the SO/FD combination were not significant for root system weight.

The lowest values seen in this study for LA, SDW, RV and RDW in the 'Pera' and 'Seleta' orange seedlings with FD as rootstock, agree with the results obtained by Rodrigues *et al.* (2016), who obtained lower values for seedlings of the 'Pera' and 'Westin' orange, the 'Piemonte' tangerine, and the 'Tahiti' acid lime grafted onto FD rootstock, in relation to the other rootstocks under evaluation.

For the PO/RL combination, the formation time of the seedlings with no interstock was 15 months from sowing the RL. The interstocked seedlings in the PO/SO/FD and PO/RL/FD combinations were obtained 17 months after sowing the FD. The seedlings with no interstock in the SO/FD combination were not ready at that time, even though the FD had been sown on the same day and under the same conditions.

FD grown alone reached grafting diameter 16 months after sowing when grafted with shoots of the 'Seleta' orange. Seedlings from this combination were ready 21

months after sowing the FD. These results indicate that the use of FD in the production of interstocked seedlings with two consecutive shoots would require a longer time for their formation, since it would still be necessary for the 'Seleta' orange to reach the minimum diameter for the second grafting with shoots of the 'Pera' orange.

The seedlings with no interstocks presented grafts and rootstocks with a single stem and a diameter of 1.23 cm, measured 5 cm above the graft line. Of the seedlings produced, 80% had a maximum deviation of 15 degrees from the graft in relation to the rootstock, meeting the standards of physical quality required for citrus seedlings according to the guidelines and standards of Embrapa (2003).

The interstocked seedlings were produced on a single stem, with diameters of 0.71 and 0.74 cm, measured 5 cm above the two grafting regions; 100% of the seedlings had a maximum deviation of 15 degrees relative to the main axis of the seedling. It is worth noting that there is no quality standard described for interstocked seedlings of citrus trees.

## CONCLUSION

Seedlings of the 'Pera' orange interstocked by nucellar tissue of the sweet orange or Rangpur Lime, using inarching as an auxiliary technique, are produced in less time than conventional interstocked seedlings, with a percentage graft success greater than 80%, and quality standards compatible with those desired for commercial production.

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