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Performance and Fruit Ripening of Red Orange Tree Grafted onto Two Rootstocks

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Abstract

The cultivar Sanguínea de Mombuca (*Citrus sinensis* [L.] Osbeck) bears lycopene-rich fruits with attractive red pulp and high nutritional value; thus, this cultivar has the potential to diversify orchards for the production of fruits for natural consumption and juice. Therefore, studies on the productive performance in combination with rootstocks are required. This study evaluated the phenological cycles, yield, ripening curve, and fruit quality of this cultivar grafted onto 'Rangpur' lime and 'Swingle' citrumelo. The study was conducted in the Midwest of the state of São Paulo, in the subtropical region of southeastern Brazil, and the field experiment was performed over two consecutive seasons. Rootstocks had little effect on the duration of the phenological cycles. The fruit ripening curve predicted the time of harvest as approximately 240 days after anthesis, regardless of rootstock. Fruits harvested from trees grafted onto 'Swingle' citrumelo showed higher levels of soluble solids and sugars and higher technological indices. The larger number of fruits and greater fresh weight confirmed that better yield performance occurred with the trees grafted onto the 'Rangpur' lime as opposed to the 'Swingle' citrumelo rootstock.

Keywords: ascorbic acid, citrus, degree-days, orange juice, phenology, soluble solids

1. Introduction

Citrus is one of the most important agricultural crops worldwide, and Brazil predominates in the production of oranges for industrial processing into juice (Domingues et al., 2021). The sustainability of citrus crops is a global concern (Tietel et al., 2020; Carvalho et al., 2021). The state of São Paulo, Brazil, is among the vulnerable regions that cultivate a reduced number of citrus genotypes, which leads to greater susceptibility to pests and diseases, as well as less economic competitiveness among growers (Carvalho et al., 2019).

Certain orange cultivars have been poorly studied and cultivated. The cultivation of red pulp oranges is an alternative for citrus orchards because of their potential for use in niche markets, such as that of juice with red pulp that naturally contains lycopene and higher concentrations of total carotenoids, or that of fresh fruits, which have appeal for their nutrition and health-related factors (Oliveira, Winkelmann, & Tobal, 2019; Saini et al., 2022).

Blood or red oranges originated in the Mediterranean region, likely from Malta or the Italian region of Sicily, and have high nutritional value (Oliveira et al., 2019). There are two subgroups of red oranges; the genuine blood orange has a high content of anthocyanins in its juice, while the juice of the red orange has a high level of carotenoids (Saini et al., 2022). Compared with regular oranges, these subgroups are less acidic, slightly sweeter, and contain more antioxidants, which darken the flesh (Russo et al., 2021).

The cultivar Sanguínea de Mombuca (*Citrus sinensis* [L.] Osbeck) arose from spontaneous mutation in the municipality of Mombuca, São Paulo State, was subsequently selected and released by the Agronomic Institute of Campinas, and currently dominates among red pulp oranges in Brazil (Latado, 2021). This cultivar is a rich source of nutraceuticals, including the carotenoids β-carotene and lycopene that are responsible for the red color of the pulp (Hamedani, Rabiel, Moradi, Ghanbari, & Azimi, 2012; Latado, 2021). The tree has early-ripening, spherical-shaped, medium-sized (140 g) fruits that contain 55% juice, on average. Due to the color of the juice, red-fleshed oranges have a higher commercial value than their orange-fleshed counterparts, while their production costs are comparable (Latado, 2021).

Grafting is a widely used technique in citrus farming (Bowman & Joubert, 2020), and when performed with the use of proper rootstock, it can provide important improvements for the scion (Balfagón, Rambla, Granell, Arbona, & Gómez-Cadenas, 2022), such as juvenile period reduction, homogeneous tree architecture, pest and disease protection, water and nutrient absorption, tolerance to abiotic stress (Rasool et al., 2020), and increased yield and fruit quality. Fruit size, juice quality, sugar and acid content, fruit skin color and thickness, and fruit ripening and production duration are also influenced by rootstock (Hu et al., 2022).

The 'Rangpur' lime (*Citrus limonia* Osbeck) tree is a natural hybrid of *Citrus medica* L. and mandarin (*Citrus reticulata* Blanco) and is suggested to be native to India (Curk et al., 2016). In Brazil, the rootstock of 'Rangpur' lime has previously been used in citrus orchards due to its compatibility with all scions, as well as its vigor, drought tolerance, high yield, precocity, and early fruit maturation (Oliveira, Mello-Farias, Oliveira, Chaves, & Herter, 2017). Although it is tolerant to *Citrus* tristeza virus (CTV), it is susceptible to *Citrus* exocortis viroid (CEVd) and *Citrus* sudden death-associated virus (SCDaV) (Fadel, Mourão Filho, Stucchi, Wulf, & Couto, 2018).

'Swingle' [*P. trifoliata* (L.) Raf × *C. paradisi* Macf.] is the most cultivated citrumelo not only in Brazil but also in the world. It is among the main rootstocks used for diversification of orange groves, providing scions with high quality fruits, high juice yield, greater SS content and yield, and lower scion vigor. This cultivar is ideal for semi-dense planting in cooler locations, and it is not compatible with all scions (Domingues et al., 2021). It is resistant to *Citrus* sudden death-associated virus and decline (Ribeiro, Nünez, Pompeu Júnior, & Mourão Filho, 2014).

Studies on phenological cycles, fruit ripening curves, and productivity have been conducted to evaluate the performance of a certain cultivar in various locations. These studies provide information for extending cultivation to other regions with similar environmental variables (Micheloud, Castro, Buyatti, & Gabriel, 2018; Carvalho et al., 2021). The search for healthier foods is increasing in terms of establishing alternative sources to improve the quality of life. Studies that examine the effects of combining red orange cultivars with rootstocks are currently relevant, since information gathered from such studies can help citrus growers to identify promising scion/rootstock combinations that can increase yield, fruit quality, and profitability (Latado, 2021).

The study grafted the Sanguínea de Mombuca cultivar onto 'Rangpur' lime and 'Swingle' citrumelo trees to evaluate the phenological variables, fruit ripening curves, and yields in the subtropical region of southeast Brazil, and the results will be useful in diversification planning for new citrus orchards, allowing the identification of the dual-market orange cultivar for industrial processing and natural consumption in the consumer markets most demanding high quality fruit

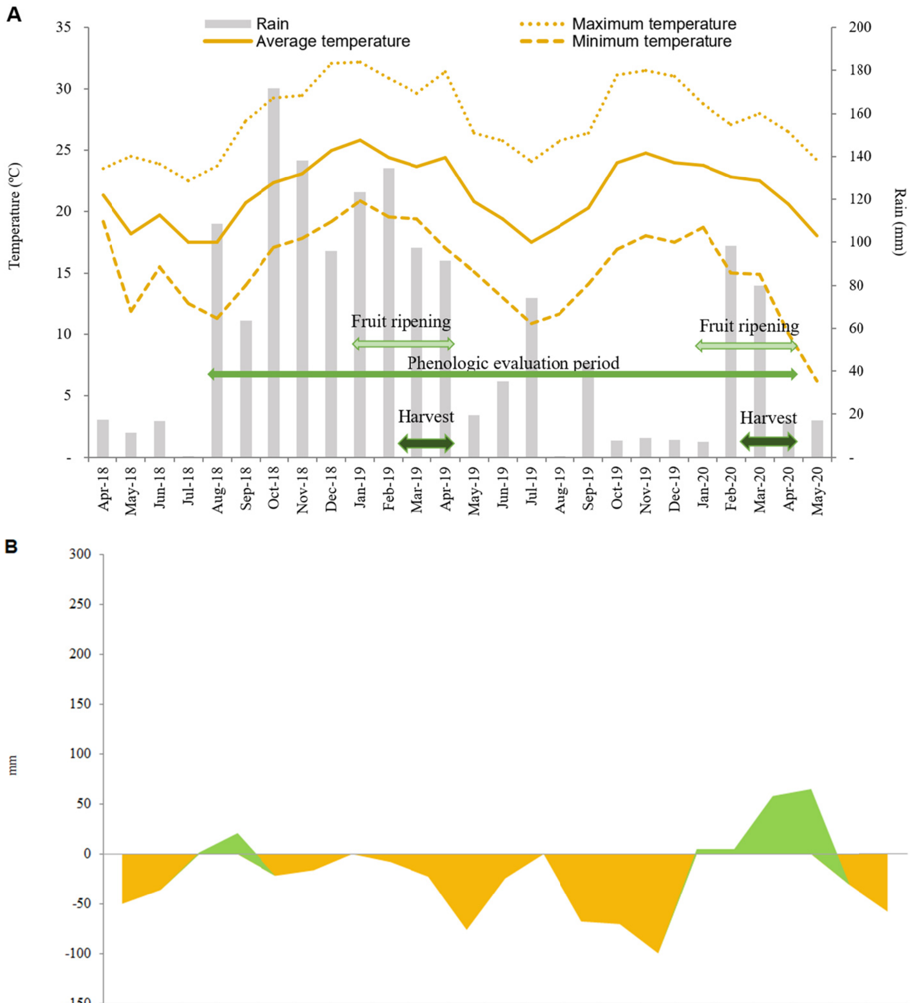
2. Materials and Methods

2.1 Experimental Area Characterization

The experiment was conducted at the São Manuel Experimental Farm, School of Agriculture, São Paulo State University (FCA UNESP), Brazil, located at 22°44′28″ S, 48°34′37″ W, at an altitude of 740 m a.s.l. According to the Köppen-Geiger climate classification, the climate of the area is *Cwa*, or warm temperate (mesothermal) and humid, and the average temperature of the warmest month is approximately 22 °C (Cunha & Martins, 2009). The soil is classified as a sandy-textured Latossolo Vermelho distroférrico according to the Brazilian system of soil classification (Santos et al., 2013), that is, a dystrophic Typic Hapludox (Soil Survey Staff, 2021).

2.2 Plant Material and Crop Management

A replicated trial was performed in two consecutive crop seasons, 2018-2020, in a non-irrigated orchard of three and four years of age, respectively. Red orange trees were grafted onto 'Rangpur' lime and 'Swingle' citrumelo trees and planted with 6 m spacing between rows and 4 m between trees (*i.e.*, 416 trees ha^{-1}). The experimental area was prepared based on soil analysis and orange crop recommendations using plowing, sorting, and liming. The trees received the standard management practices recommended for citrus orchards. A weather station located 100 m from the experimental area recorded daily precipitation (mm) and maximum, minimum, and average temperatures (°C) throughout the experimental period, and the water balance is shown in Figure 1.



	Aug	Sep	Out	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Out	Nov	Dec	Jan	Feb	Mar	Apr
EXC	0	0	2	21	0	0	0	0	0	0	0	0	0	0	0	5	5	58	65	0	0
DEF	-50	-36	0	0	-22	-16	0	-8	-23	-76	-24	0	-68	-70	-100	0	0	0	0	-31	-58

Figure 1. (A) Precipitation, average, maximum and minimum monthly temperatures, during phenological study period, fruit ripening and harvest time; (B) Climatic water balance

2.3 Treatments and Experimental Design

The treatments consisted of two scion/rootstock combinations: 'Sanguínea de Mombuca'/'Rangpur' lime (SM/RL) and 'Sanguínea de Mombuca'/citrumelo 'Swingle' (SM/SC). The experimental design was a randomized complete block with two treatments and five replicates. Each replicate consisted of three trees per experimental plot, with border trees external to the trial.

2.4 Phenological Cycles

Six branches per tree were randomly chosen and marked in the mid-section of the graft around the circumference. The phenological study started by evaluating the following stages: (3) floral opening (anthesis); (4) dried petals and stylet; (5) without petals or stylet; (6) fruits 3 cm in diameter; (7) fruits 4.5 cm in diameter; (8) unripe fruits approaching final size; (9) fruits changing color from green to yellow; and (10) ripeness index or ratio (soluble solid/titratable acidity) between 8.5 and 10 (Barbasso, Pedro Júnior, & Pio, 2005) (Figure 2).

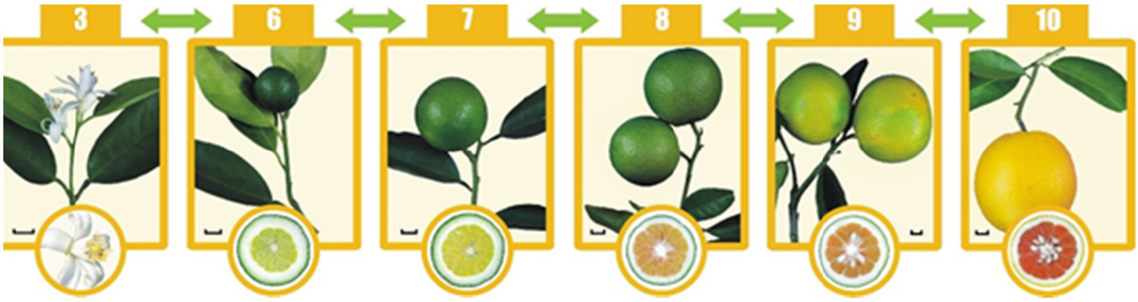


Figure 2. Rating scale designed for the phenological stages of trees

The branches were reviewed every 20 days, and grades of 0-10 were issued. These scores were calculated when 51% of the branches were at the appropriate phenological stage.

The intervals between the stages were evaluated for the phenological characterization of the scion/rootstock combinations. Each phenological interval was measured in days and was calculated as total degree days (DDs), according to the models of Ometto (1981).

2.5 Yield

The total number of fruits harvested from each tree were counted, and the product of the total number of fruits and fresh weight of the fruits harvested per tree was considered the production per tree, and was expressed in kg $tree^{-1}$. The number of boxes per tree was calculated as the ratio of the production to the standard box weight of 40.8 kg; the yield ($t\ ha^{-1}$) was obtained considering a stand of 416 trees per hectare.

The canopy volume (m^3) of each tree was estimated using Equation 1 (Zekri, 2000).

$$\text{Canopy volume} = \pi r^2 \cdot h \quad (1)$$

Where, r = canopy radius; h = tree height.

Production efficiency ($kg\ m^{-3}$) was determined using the relationship between production per tree (kg) and canopy volume (m^3) (Equation 2).

$$PE = PT/CAV \quad (2)$$

Where, PE = production efficiency; PT = production per tree; CAV = canopy volume.

2.6 Fruit Ripening and Quality

Evaluations of the fruit-ripening curve started 180 days after anthesis (DAA) and ended when the fruits presented a ratio between 8.5 and 10. During this period, five fruits per tree were harvested at 20-day intervals.

The average fruit weight (g) was obtained by weighing the fruits on a semi-analytical balance; length and diameter (mm) were obtained by measuring the longitudinal and equatorial diameters of the fruits using a digital caliper; juice yield was calculated as a percentage of the juice weight/fruit weight ratio and the results were expressed as percentages.

A colorimeter was used to assess the skin and pulp color. A texturometer with an SMS P/2 compression probe was used to measure firmness by compressing the fruit 10 mm from the point of contact at a speed of 1.0 mm s^{-1} (N).

Titratable acidity (TA) was determined by titration with 0.1 N sodium hydroxide (NaOH) in a solution of 10 mL of juice, 50 mL of distilled water, and 0.3 mL of phenolphthalein, and expressed as a percentage of citric acid. Soluble solids (SS) were measured with a digital refractometer using three drops of juice and expressed in Brix. The ripeness index or ratio was calculated as the relationship between the soluble solids content and the titratable acidity (Ribeiro et al., 2020).

The technological index (TI) was calculated using the equation proposed by Di Giorgi et al. (1990) and expressed in kg SS box^{-1} (Equation 2):

$$\text{Technological index} = (JY \times SS \times 40.8)/10{,}000 \quad (2)$$

Where, JY = juice yield (%); SS = soluble solids (°Brix); 40.8 = standard weight of the box used to harvest oranges (kg).

The reducing and non-reducing sugar content in the juice was estimated using the method of Lane and Eynon (1923), as described by Anjum, Akram, Zaidi, and Ali (2020).

Ascorbic acid content was determined according to the Association of Official Analytical Chemists method 967.21 (AOAC, 2019). The homogenized juice extract (10 mL) was diluted in 100 mL of 3% metaphosphoric acid and passed through filter paper. Next, 5 mL of the filtrate was titrated with 2,6-dichlorophenol iodophenol (DCPIP) as the indicator. The results are expressed in mg 100 g^{-1}. A standardization curve was created by titration with standard solutions containing a known amount of ascorbic acid

2.7 Statistical Analysis

The two-year data were analyzed using repeated measures, and the reported data are the mean of the two years of evaluation. Data were subjected to analysis of variance, and when significance was established by the F test, the Tukey test was used to compare the rootstock means. To evaluate the ripening curve, a split-plot design was used, with the rootstocks (plots) compared by the Tukey test and the DAA (subplots) evaluated by regression analysis. Analysis of variance was performed using the AgroEstat® software and the regression analysis using the SISVAR software.

3. Results

3.1 Phenological Cycles

The SM/RL combination accumulated 962.83 DDs and remained for 77 days in phenological range 6-7, while the SM/SC combination accumulated 1359.55 DDs and remained for 100 days. This phenological interval was the longest in this study (Figure 3A), and also in that by Nascimento, Ribeiro, Bastos, Ferreira de Sá, and Nascimento (2018). The phenological interval 8-9 occurred for 24 days and accumulated 318.76 DDs for fruits from the SM/SC combination, and 38 days and 498.76 DDs for the SM/RL combination (Figure 3A). This phenological interval corresponds to the stage of skin color change caused by chlorophyll degradation and carotenoid synthesis, which are regulated by the environment, soil, rootstock, carbohydrates, and nitrogen compounds (Domingues et al., 2021).

The rootstocks only influenced ($p < 0.01$) the number of days, and the DDs accumulated in phenological interval 6-7 (fruits of 3-4.5 cm diameter) and 8-9 (unripe fruits approaching final size; fruits changing color from green to yellow) (Figures 3A and 3B). Neither rootstock had a significant effect on the total duration of the phenological cycles of red oranges. The total cycle had an average duration of 246 days, with an accumulation of 3226.72 DDs, regardless of the scion/rootstock combination. The total number of DDS for each cultivar, from flowering to harvest, was similar, and citrus flowering occurred between August and October in both crop seasons. The data indicated that the rootstocks had no influence, as full flowering occurred during the same period for both combinations.

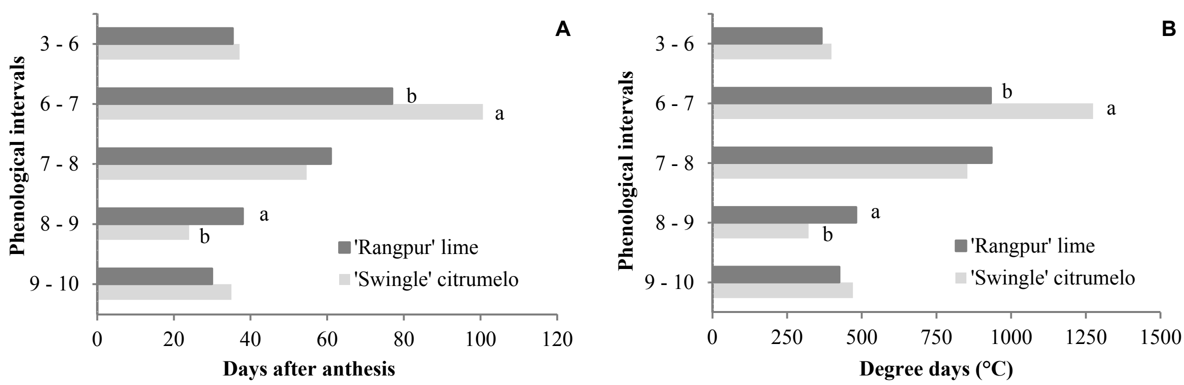


Figure 3. Phenological intervals of the 'Sanguínea de Mombuca' orange tree measured in days and degree days onto two rootstocks. Columns followed by different letters within each range are significantly different by Tukey test at 5% probability

3.2 Yield Performance

Scion/rootstock combinations had a significant effect on the number of fruits, production per tree, yield, and production efficiency. However, scion/rootstock combinations did not have a significant effect on canopy volume (Table 1). The SM/RL plants outperformed the SM/SC trees in terms of fruit number, production per tree, yield, and production efficiency.

Table 1. F-test values and means of fruit number and production per tree, yield, canopy volume and production efficiency of red orange grafted onto two rootstocks

Variation sources	No. of fruits per tree	Production (kg per tree)	Yield (t ha^{-1})	Canopy volume (m^3)	Production efficiency (kg m^{-3})
	---------- F-test values ----------				
Block	1.38^{ns}	2.51^{ns}	2.51^{ns}	2.19^{ns}	2.51^{ns}
Rootstock	9.03^{**}	59.77^{**}	59.79^{**}	1.28^{ns}	50.62**
C.V. (%)	22.27	10.67	10.67	17.66	19.87
Rootstock	---------- Means ----------				
'Rangpur' lime	125.71a	23.43a	9.770a	1.95a	12.18a
'Swingle' citrumelo	89.68b	13.97b	5.823b	2.15a	5.82b
Overall mean	107.69	17.60	7.336	2.05	8.99

Note. ns = not significant; * = significant at 5%; ** = 1% significant by F test. Equal column letters do not differ by Tukey test at 5% probability level.

3.3 Fruit Ripening and Quality

Days after anthesis (DAA) had an effect on red orange fruit diameter and length, whereas rootstocks had no effect. There was a quadratic increase in both variables. The maximum diameter (71.1 cm) and length (65.2 cm) were observed at 247 DAA (Figures 4A and 4B). The onset of the dry season was responsible for the decrease in both variables at 260 DAA (April to May; Figure 1). Despite these variations, the red orange met the requirements of the Brazilian fresh fruit market as a middle-class fruit measuring 65-71 mm in diameter.

There was a significant interaction between scion/rootstock and DAA combinations for fruit weight ($p < 0.01$). A quadratic model was used to adjust the progression of fruit weight along the DAA. The weight increase was similar between the two combinations up to 220 DAA. The maximum weight (161.1 g) was reached in the SM/SC combination at 242 DAA and in the SM/RL combination at 258 DAA (187.7 g; Figure 4C).

There was no significant interaction for juice yield, although there was an isolated effect of scion/rootstock and DAA combination. The average juice yield of the SM/SC combination was 44.43% compared to 39.71% for the SM/RL combination, despite the latter promoting greater fruit weight. Both combinations showed a quadratic increase in juice yield when measured as a function of DAA, reaching a maximum of 46.2% at 227.4 DAA (Figure 4D). Fruit firmness was affected only by DAA ($p < 0.01$) and was quadratically reduced during the ripening period (Figure 4E).

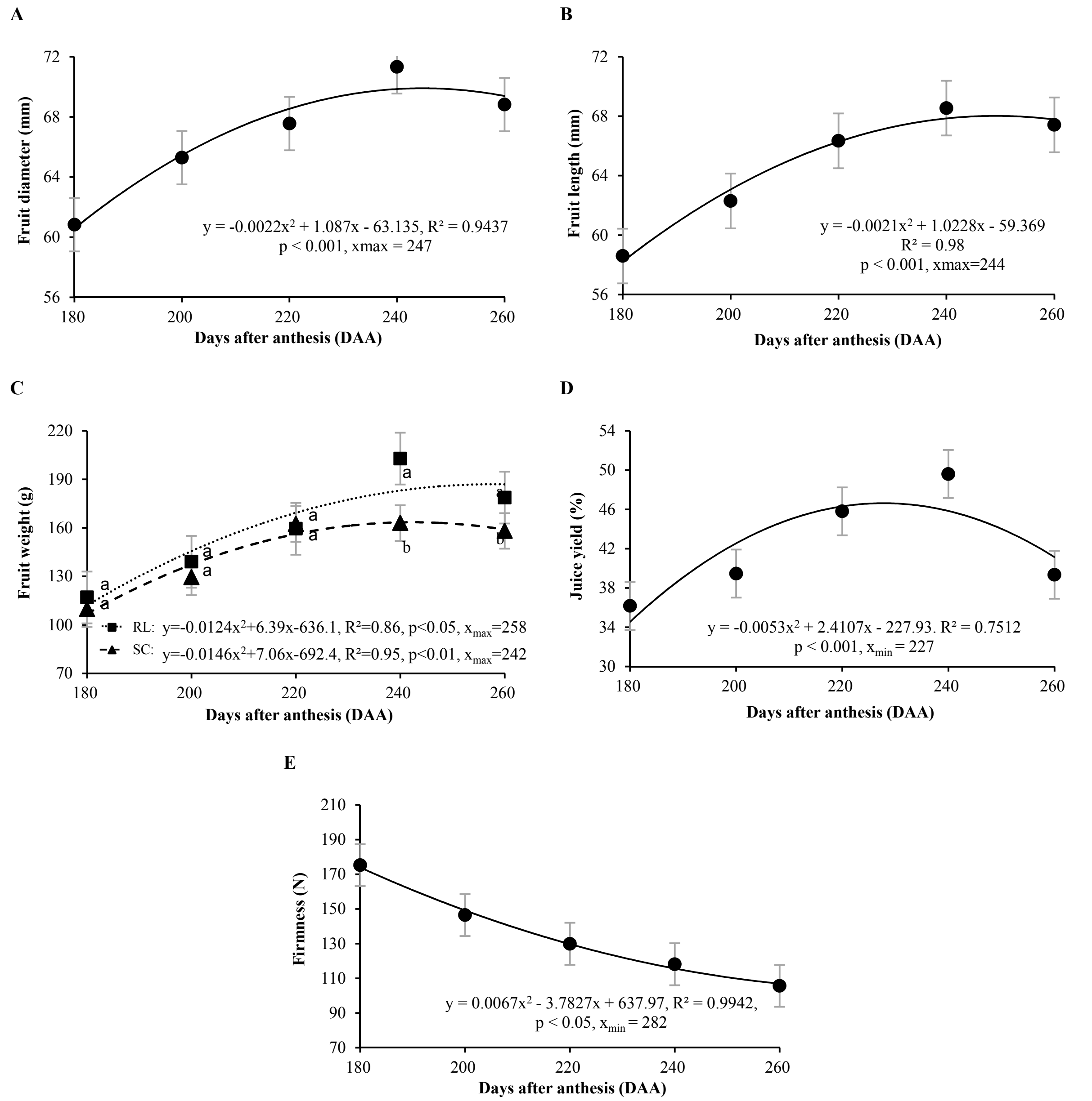


Figure 4. Fruit diameter (A), length (B), weight (C), juice yield (D) and firmness (E) of the fruits of red orange grafted onto two rootstocks, as a function of fruit collecting stages. The Tukey test at the 5% probability level finds that points followed by distinct letters are different in each DAA. Data in figures A, B, D and E are the mean of the two rootstocks

The luminosity and chromaticity varied only as a function of the DAA, with a positive linear effect for both combinations, compared to those indicated for the skin color (Figures 5A and 5B). The fruits changed from intense green to light yellow, according to the time of data evaluation. The DAA and scion/rootstock combinations had significant interactions with the skin hue angle (°h) ($p < 0.01$). The mean values for the SM/SC and SM/RL combinations decreased quadratically and linearly, respectively (Figure 5C). Based on the observed values, the fruits of 'Sanguínea de Mombuca' in both combinations were predominantly green at the beginning of the evaluations, but showed a greater intensity of the yellow component at the end of the evaluations, especially in the fruits of the SM/SC combination, which showed the lowest °h.

There was no significant interaction between the variables in terms of pulp color, luminosity, chromaticity, or °h. However, there was an isolated effect of the scion/rootstock combinations ($p < 0.05$) on luminosity, and an isolated influence of DAA ($p < 0.01$) on chromaticity, but there was no effect of any component on hue angle

(°h). The SM/SC combination produced fruits with the highest average juice luminosity (56.63). The juice from the fruits of the SM/RL combination had a luminosity of 53.61. The average juice hue angle was 48.23. A juice hue value equal to 0 ºh is equivalent to red, and 90 ºh is equivalent to yellow. The color spectrum of the red orange juice is an intermediate between the two, regardless of the rootstock. Pulp color intensity increased until harvest, indicating a positive linear effect on chromaticity throughout the ripening period (Figure 5D).

There was no significant interaction between the scion/rootstock combinations and the DAA for soluble solids, titratable acidity, or ripeness index. The scion/rootstock and DAA had an isolated effect ($p < 0.01$) on the first two variables, while ripeness index only had a significant effect on DAA ($p < 0.01$). The fruits of the SM/SC combination presented the highest mean soluble solids (8.71 °Brix) and titratable acidity (0.98%). The SM/RL combination produced fruits with 7.93 ºBrix and 0.88% acidity. Soluble solids increased linearly with DAA concentration (Figure 6A). A quadratic decrease in titratable acidity was observed, with a minimum acidity value (0.7% citric acid) at 239 DAA (Figure 6B). The ripeness index increased quadratically along the DAA, reaching an estimated maximum value of 10.4 at 240 DAA (Figure 6C).

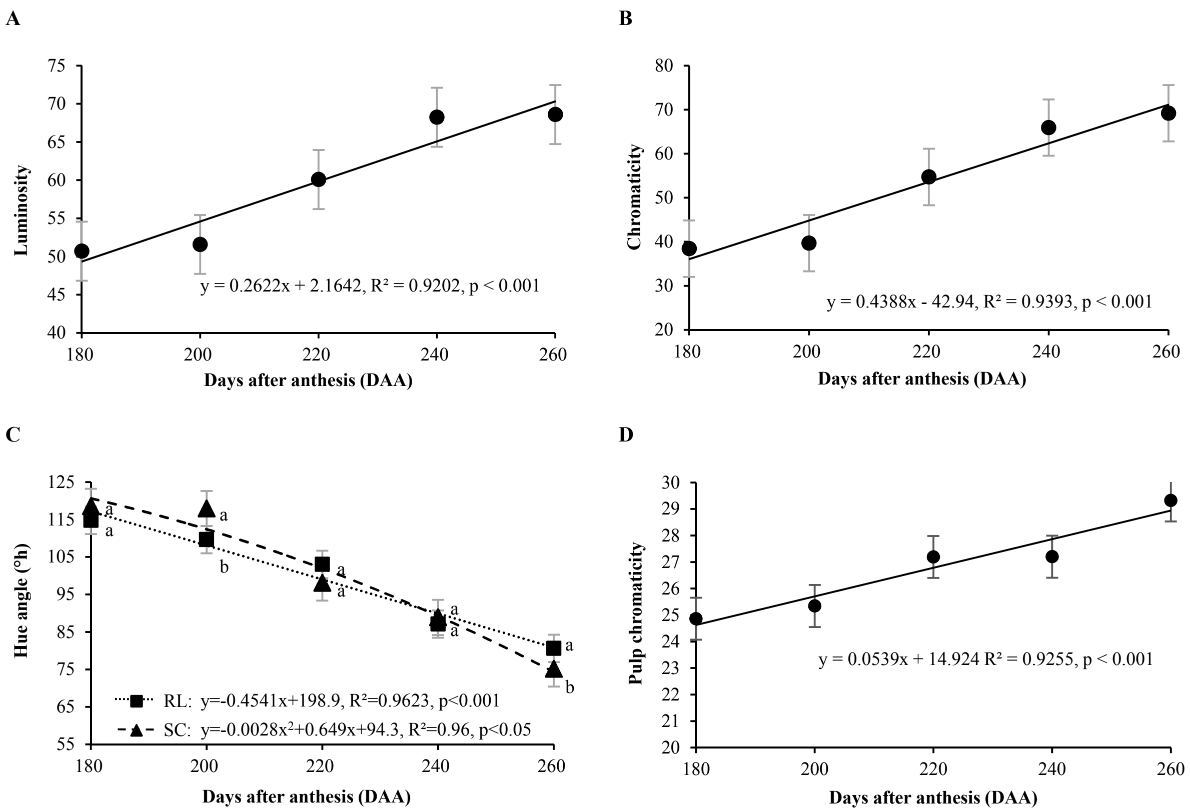


Figure 5. Luminosity (A), chromaticity (B), and hue angle (C) of the skin, and chromaticity of the pulp (D) of red orange grafted onto two rootstocks, as a function of fruit collecting stages. The Tukey test at the 5% probability level finds that points followed by distinct letters are different in each DAA. Data in figures A, B and D are the means of the two rootstocks

The scion/rootstock and DAA combinations showed a significant interaction ($p < 0.01$) for the technological index. Both scion/rootstock combinations showed a quadratic fit. At 246.7 DAA, the SM/SC combination had a maximum value of 1.8 kg SS box^{-1}, while the SM/RL combination had a maximum of 1.2 kg SS box^{-1} at 221.4 DAA. During the fruit ripening period, the SM/SC combination showed increased fruit development with a higher technological index (Figure 6D).

A B

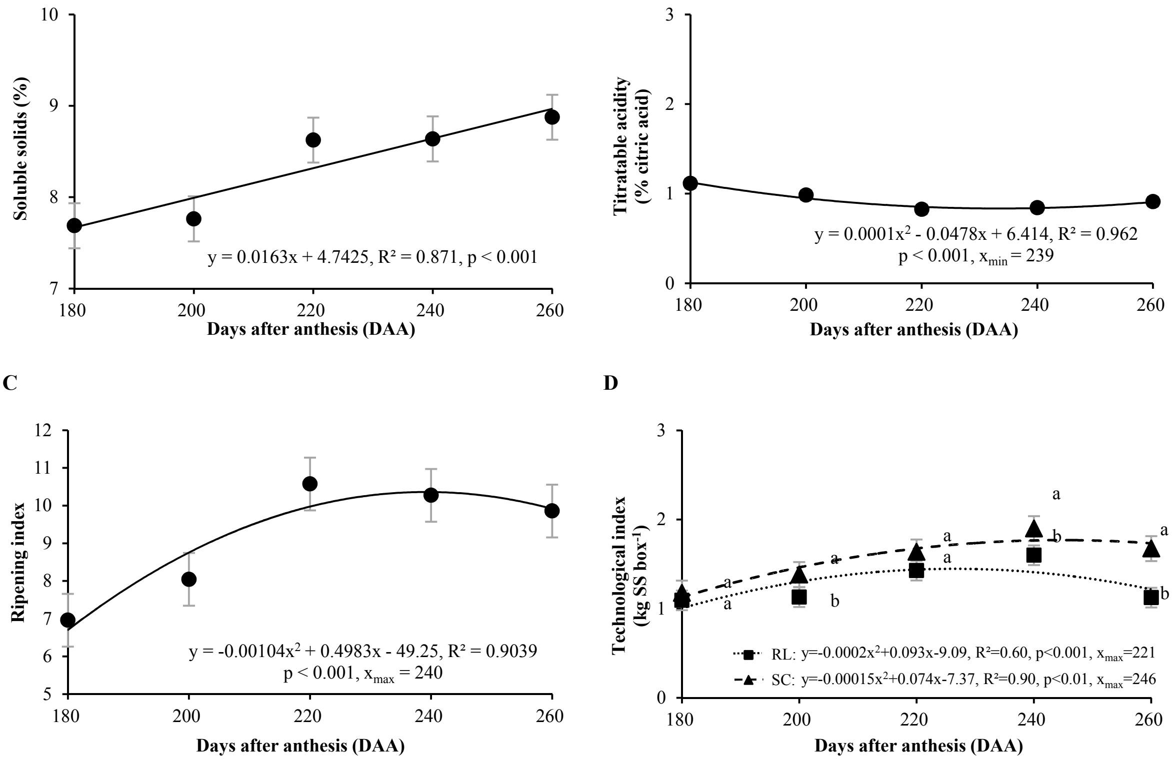


Figure 6. Soluble solids (A), titratable acidity (B), ripeness index (C) and technological index (D) of red orange grafted onto two rootstocks, as a function of fruit collecting stages. The Tukey test at the 5% probability level finds that points followed by distinct letters are substantially different in each DAA. Data in figures A, B and C are the means of the two rootstocks

There was an interaction between the scion/rootstock and DAA combinations ($p < 0.01$) for the concentration of ascorbic acid (AA). In SM/RL, the AA concentrations of the fruits decreased quadratically, reaching a minimum point at 222 DAA (Figure 7A). Regression models (linear and quadratic) for the SM/SC combination did not fit the available data.

There was a significant interaction between the scion/rootstock and DAA combinations for reducing and total sugars ($p < 0.01$ and $p < 0.05$, respectively), and an isolated effect of DAA on non-reducing sugars ($p < 0.01$). The content of reducing sugars in SM/SC fruits increased linearly during ripening and was higher at 260 DAA than that of SM/RL fruits. The SM/RL combination showed a quadratic decrease in reducing sugar content from 222 DAA (Figure 7B). However, these fruits reached their maximum weights at 258 DAA. Data from both scion/rootstock combinations were fitted to a quadratic model for the percentage of total sugars. At 258 DAA, the maximum total sugar content (6.10%) was obtained for the SM/SC combination. The SM/RL combination reached 4.5% at 236 DAA (Figure 7C). There was a quadratic increase in the means of non-reducing sugars up to 239 DAA, with a maximum value of 2.3% (Figure 7D).

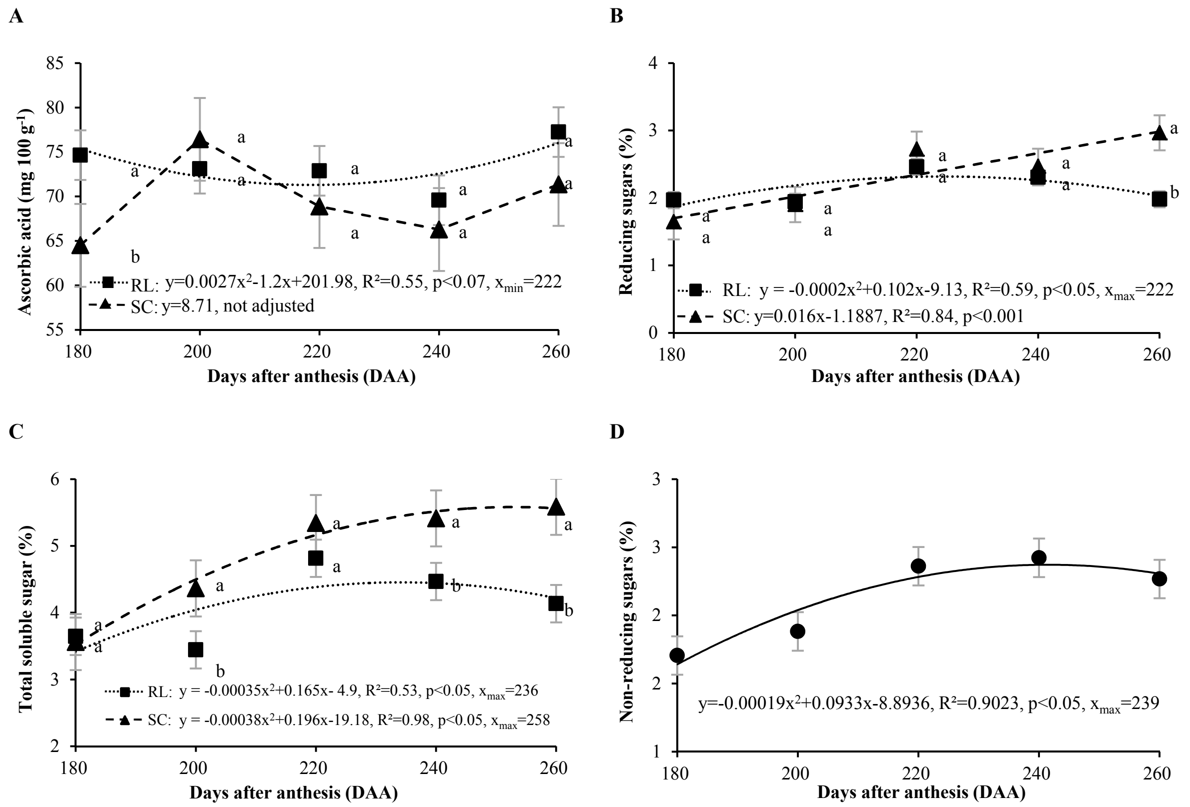


Figure 7. Ascorbic acid content (A), percentage of reducing (B), total (C) and non-reducing (D)sugars in red orange fruits grafted onto two rootstocks, as a function of fruit collecting stages. The Tukey test at the 5% probability level finds that points followed by distinct letters are substantially different in each DAA

4. Discussion

The vegetative growth of citrus trees occurs during seasonal cycles or flushes throughout the year. Flushes are normally more intense in spring, less intense in early autumn, and absent in winter. Leaf flushes create large spikes in phytohormone levels in the trees, which can affect growth, yield, and quality (Marks, 2022). Temperature and soil moisture are the main environmental variables that regulate flushing. Major new shoots emerging in late spring and early summer, coinciding with increase in temperature and water availability, were observed in São Paulo State in Brazil (Carvalho et al., 2021), as well as in Florida and California (Kistner, Amrich, Castillo, Strode, & Hoddle, 2016) in the USA.

The data obtained in this experiment confirm these reports and reinforce the precocity of RL in relation to SC, which is reported as the first and greatest flushing of RL. Tree growth in SC is less vigorous than that of RL (Ribeiro et al., 2014; Cruz et al., 2019). The lower number of days and accumulated DDs of the SM/RL combination can be attributed to various hypotheses, including that of increased efficiency in water absorption by the RL accelerating fruit growth (Prieto, Armas, & Pugnaire, 2012). Nascimento et al. (2018) evaluated the phenology of different citrus cultivars in the northeast region of Brazil. The precocious nature of 'Rangpur' lime was also observed when compared to 'Volkamer' lemon (*Citrus volkameriana* Ten. & Pasq.).

Information on the communication between roots and shoots and the modifications by the rootstock to scion physiology and metabolism is limited (Balfagón et al., 2022). Under drought stress, some rootstocks were able to transfer their survival strategy to the scion by modulating the accumulation of hormones and antioxidant compounds (Balfagón et al., 2022).

The yield of citrus crops is directly related to the level of photosynthesis; therefore, maintaining strong levels of this process to enhance crop growth is crucial. The leaves of citrus trees have a limited period when they are able to photosynthesize and once they reach 40-50 days of age, they lose most of their ability to perform this activity, and if new leaves do not develop, the tree will not be able to produce enough carbohydrate reserves to sustain itself. Leaf flushes allow citrus trees to maintain photosynthesis at adequate levels. To develop new leaves, the tree must produce new cells, and this cell division is powered by the growth hormone auxin. Leaf flush is thereby accompanied by a large increase in auxins, which are synthesized in the foliage (Marks, 2022).

The differences between the rootstocks evaluated with higher RL yield can be attributed to the quantity and horizontal and vertical distribution of roots and radicles, the efficiency of water absorption and transport, and the opening and closing of leaf stomata (Castle, 2010). According to Balfagón et al. (2022), the differences between citrus rootstocks are partly due to a higher antioxidant capacity, better stomatal regulation that allows leaf cooling, and accumulation of protective proteins.

Both rootstocks had a comparable effect on the canopy development of the red orange tree. Knowledge of the impact of rootstock on vegetative growth of citrus tree canopies is important, as it can be used to program the planting density of new orchards and subsequent cultural practices, such as pruning, fertilizing, and harvesting (Carvalho et al., 2021). The better production efficiency of the SM/RL trees was a function of the higher yield since there were no changes in scion volume, and the overall flushing patterns were similar for both combinations. However, the advantage of the SM/RL combination in relation to these factors might be related to the crop management used in this study. The SC rootstock has a medium tolerance to water shortages, which rendered it more susceptible to unfavorable conditions in the two crop seasons evaluated (Figure 1), which affected the performance of the SM/SC combination (Ribeiro et al., 2014).

The same environmental conditions interacted differently depending on the scion and rootstock combination (Carvalho et al., 2021). Water availability, rootstock, evaporation, soil type, and temperature are the factors that most influenced the development, production, fruit number, and productivity of citrus trees (Machado, Machado, Machado, & Ribeiro, 2010). Under favorable temperatures, citrus can be forced to grow at almost any time by manipulating the water supply (Teodoro et al., 2020). This suggests that, depending on the rootstock used, irrigation may be beneficial, as with SC, as water deficit can have a major impact on fruit setting, output, and quality (Nascimento et al., 2018).

França Amorim, Girardi, Passos and Soares Filho (2016) reported that the production of trees grafted onto RL was higher than that of those grafted onto SC in a dry land system using the ‘Valencia Tuxpan’ orange tree. Teodoro et al. (2020) concluded that the ‘Sunki Tropical’ mandarin had high cumulative yields when compared to the ‘Cravo Santa Cruz’ lime (common name Rangpur lime), both grafted on the “Jaffa” orange tree. However, Domingues, Neves, Yada, Pereira Leite Júnior, and Tazima (2018) found no variations in yield performance between rootstocks of the ‘Cadenera’ orange tree grafted on RL or SC. These reports reinforce the results obtained in the present study highlighting that rootstock selection had a direct impact on tree yield, especially when distinctive features are considered as well as growing conditions.

The ripening of orange fruits is strongly influenced by the climatic conditions of the growing region (Domingues et al., 2021). Rootstocks can affect fruit quality through different biological processes, such as the induction of gene expression, change in enzyme activities (Tietel et al., 2020), change in hormone levels, and expression and transport of miRNAs and proteins (Hu et al., 2022). The rootstock plays an important role in fruit ripening because it can accelerate or delay citrus tree development (Domingues et al., 2021).

The effects of rootstock on fruit quality can be attributed to several factors, such as nutrient absorption and transport, compatibility, hormonal signaling, and gene expression (Tietel et al., 2020). The greater fruit weight produced by SM/RL at 258 DAA may be attributed to the robustness of this rootstock, as it has a deep root system that allows greater absorption of water and nutrients (Prieto et al., 2012). Heavier fruits correspond to increasingly diluted organic acids (Carvalho et al., 2020). Therefore, fruits with higher albedo and flavedo and lower juice yield will present fewer carbohydrates, acids, amino acids, and vitamins in the juice (Béber et al., 2018).

These factors may explain the results obtained, as the SC rootstock favored the development of smaller fruits. Similarly, Petry, Reis, Silva, Gonzatto, and Schwarz (2015) found that the average mass of ‘Monte Parnaso’ orange fruit grown on the RL rootstock was greater than that grown on the SC rootstock. Cruz et al., (2019) reported the opposite while analyzing the quality of ‘Navelina’ orange fruit grown on these rootstocks, indicating that this is a genotype-dependent variable.

Beber, Álvares, and Kusdra, (2018) also observed a reduction in fruit mass, along with a decreased diameter and length in the dry region. However, withdrawal of irrigation periods had little effect on the average fruit mass produced by lemon trees in two Tzaneen citrus orchards, where warm and dry conditions prevailed (Van Den Berg, 1986). These results reinforce the need for research in different regions, since environmental conditions have a direct effect on citrus yield.

'Swingle' citrumelo is among the main rootstocks used in the diversification of orange groves, as it provides the scions with high quality fruits, higher juice yield and SS content, and reduced scion vigor, as well as producing thin-skinned fruits (Castle et al., 2010; Pompeu Júnior & Blumer, 2011). Thinner skins relate to higher juice yields (Légua, Bellver, Forner, & Forner-Giner, 2011). These reports explain why the fruits from the SM/SC combination showed a higher juice yield.

Carvalho et al. (2020) also found that the fruits of 'Salustiana' orange trees grafted onto SC produced more juice than those grafted onto RL. According to Domingues et al. (2021), when grafted onto *Poncirus trifoliata* hybrid rootstocks, orange trees produced fruits with higher SS content and juice yield. Juice yield is a variable that can be used in the planning of new orchards to produce high-quality fruits with desirable features for the orange juice industry (Domingues et al., 2021).

The ripening of citrus fruits passes through different phenological stages, and the final stage is characterized by a reduced growth rate and changes in skin color (Domingues et al., 2021). Thermal amplitude with hot days (20-25 ^{o}C) followed by cool nights (10 ^{o}C) favors fruit pigmentation as it stimulates the synthesis of carotenoids and the breakdown of chlorophyll (Balfagón et al., 2022). Temperatures above 20 °C were recorded during the fruit ripening period (180-260 DAA) (Figure 1), favoring skin color. The data obtained explained the greater pigmentation of the dominant yellow fruit. During the ripening stage, the color of the juice can change because of variations in pigment concentrations. This is attributable to the fact that the water content of fruits increases as they ripen. This finding can be explained by the greater juice yield found in SM/SC combination fruits, since higher juice yields correspond to greater concentrations of pigments, acids, carbohydrates, and vitamins in the juice (Béber et al., 2018).

Although the external and internal ripening of orange fruits generally coincide, the skin and pulp behave in many respects as separate organs and can be considered to undergo different physiological processes. Ripe citrus pulp contains a large percentage of water (85-90%) and many different constituents, including carbohydrates, organic acids, amino acids, vitamin C, minerals, and small amounts of lipids, proteins, and secondary metabolites such as carotenoids, flavonoids, and volatiles (Hamedani et al., 2012). The reduction in fruit firmness over the evaluation period was related to the solubilization of pectic compounds or the change from insoluble pectin to soluble pectin as fruit ripening progressed. These insoluble compounds are found in unripe fruits and are composed of carboxylic acids bound to calcium, resulting in calcium pectate (Légua et al., 2011).

The increase in the percentage of soluble solids and decrease in titratable acidity in the fruits caused an increase in the ripeness index, which is used to determine fruit maturation (Carvalho et al., 2020). Since the technological index includes both fruit weight and soluble solids, the outcome of this variable is affected by the factors that influence the performance of these parameters (Volpe, Schöfef, & Barbosa, 2002). A decrease in juice yield translates into a decline in technological index (Di Giorgi et al., 1990). Scions on trifoliate orange rootstocks and their hybrids as SC could produce better quality fruits than those on other commonly used rootstocks (Hu et al., 2022). According to those authors, this phenomenon has been well documented, but the genetic factors affecting fruit quality through the interaction of the scion and rootstock remain unclear. The results demonstrated consistent correlations with fruit quality of four cultivars grafted onto *Poncirus trifoliata* rootstocks related to the differential expression of small RNAs.

The data showed that the decrease in AA as a function of evaluation stage may have been influenced by the climate, since rain fell from March onwards and temperatures remained high, resulting in increased concentration due to the loss of water in the fruit. There were periods of severe drought during the assessment period (Figure 1). As AA biosynthesis is generated from D-glucose, with nucleotides and sugars as intermediates, this scenario, together with the fact that the SC rootstock presents lower tolerance to water deficit, which can limit the photosynthetic capacity of the tree, may explain these results (Loannidi et al., 2009).

Another possible explanation is that variations in AA concentrations may occur during fruit development in response to different stresses. AA was oxidized and converted to dehydroascorbic acid at this stage, which may explain the higher and lower concentrations of AA during the evaluations. Photosynthesis, temperature, and light exposure can affect AA synthesis and production (Lee & Kader, 2000). Environmental factors such as irradiation and stress can stimulate the expression of genes involved in the production of AA (Loannidi et al., 2009).

The contents of reducing sugars in the SM/SC fruits were higher than those in the SM/RL combination, because the SM/RL showed a quadratic decrease in the content of reducing sugars. The concentration effect, represented by fruit size, may explain this decrease. The fruits grew throughout this period, and carbohydrates were translocated to all parts of the fruits, including the juice, albedo, and flavedo. The increase in the total sugar concentration occurs because the sucrose concentration increases during the entire growth and ripening phases of the fruit (Carvalho et al., 2020). This intensity is intrinsically linked to the photosynthetic process, which can be affected by climatic conditions and the rootstock (Volpe et al., 2002).

The ripening of citrus fruits passes through different phenological stages, and the final stage is characterized by a reduced growth rate, an increase in soluble solid content, a decrease in titratable acidity, and changes in skin color (Domingues et al., 2021). The harvest quality and optimal citrus harvest time are based on the SS content and TA, as well as their relationship (the ripeness index or ratio: SS/AT). The ripeness index or ratio represents the balance between the sugar and organic acid contents in the fruits; it is associated with juice taste, and is widely used in the orange juice industry as an indicator of ripening and fruit quality (Domingues et al., 2021).

In this study, precipitation and temperature were responsible for the reduction in juice yield, non-reducing sugars, and titratable acidity after 239 DAA. Precipitation decreased after March for both crops evaluated, but temperatures remained high (Figure 1). Although there was a linear increase in soluble solid content, this may have been due to the effect of concentration, that is, the loss of water in the fruits. The increase in titratable acidity after 239 DAA can be similarly explained. Therefore, harvesting red orange fruits at approximately 240 DAA is ideal for generating the maximum use of the raw material.

5. Conclusions

Red orange 'Sanguinea de Mombuca' grafted on rootstocks of 'Rangpur' lime and 'Swingle' citrumelo completed the cycle from anthesis to harvest in 246 days, with an accumulation of 3226.72 degree-days. The best qualitative attributes were obtained at approximately 240 days after anthesis for the trees grafted onto the two rootstocks. The combination 'Sanguínea de Mombuca/'Swingle' citrumelo presented fruits with higher contents of soluble solids and total and reducing sugars, and a greater technological index at the end of ripening, while the combination 'Sanguínea de Mombuca'/'Rangpur' lime presented a higher number of fruits with greater weight and better yield performance. The 'Rangpur' lime rootstock stood out for its better yield performance due to its higher drought tolerance. 'Swingle' citrumelo stands out for improving the fruit quality of the red orange. The choice of the most appropriate rootstock should take into account the profitability of the citrus grower and the type of consumer market.

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