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Trifoliate Orange-Related Rootstocks Enhance the Horticultural Performance of 'Shamouti' Sweet Orange under Humid Subtropical Condition

Deived Uilian de Carvalho [1,2,3,*], Rui Pereira Leite Junior [1], Inês Fumiko Ubukata Yada [4] and Zuleide Hissano Tazima [5]

[1] Área de Proteção de Plantas, Instituto de Desenvolvimento Rural do Paraná–IAPAR/Emater (IDR-Paraná), km 375 Celso Garcia Cid Road, Londrina 86047-902, PR, Brazil
[2] Centro de Ciências Agrárias, Universidade Estadual de Londrina (UEL), km 380 Celso Garcia Cid Road, Londrina 86057-970, PR, Brazil
[3] Departamento de Pesquisa e Desenvolvimento, Fundo de Defesa da Citricultura (Fundecitrus), 201 Dr. Adhemar Pereira de Barros, Araraquara 14807-040, SP, Brazil
[4] Área de Biometria, Instituto de Desenvolvimento Rural do Paraná–IAPAR/Emater (IDR-Paraná), km 375 Celso Garcia Cid Road, Londrina 86047-902, PR, Brazil
[5] Área de Fitotecnia, Instituto de Desenvolvimento Rural do Paraná–IAPAR/Emater (IDR-Paraná), km 375 Celso Garcia Cid Road, Londrina 86047-902, PR, Brazil
* Correspondence: deived.carvalho@fundecitrus.com.br

Abstract: The narrow genetic pool for both scions and rootstocks used in the Brazilian orchards makes the citrus industry vulnerable to biotic and abiotic threats. Orchard diversification by using different scion–rootstock combinations is an efficient measure to promote citrus protection, through increasing the level of genetic diversity. In this paper, we report the horticultural performance of the mid-season 'Shamouti' sweet orange grafted on five different rootstocks ('Rangpur' lime, 'Swingle' citrumelo, 'C-13' citrange, and 'Cleopatra' and 'Sunki' mandarins) in a long-term experiment (2007–2017) under the Brazilian humid subtropical condition. 'Shamouti' trees were assessed for vegetative growth, yield, and fruit quality. Additionally, a study was performed to estimate tree density and yield for new plantings. Trees grafted on 'Swingle' and 'C-13' rootstocks were less vigorous and more productive, with cumulative yields of >480 kg per tree, allowing high-density plantings (363–337 trees·ha^{-1}). Trees on 'Cleopatra', 'Sunki', and 'Rangpur' were the most vigorous among the tested rootstocks, with tree heights > 4.20 m. However, they took longer to establish in the field, evidenced by their growth progress. These combinations also displayed the lowest tree density estimation ($\leq$311 trees·ha^{-1}). Trees on 'Cleopatra' exhibited the lowest cumulative yield (255 kg per tree). Although some significant differences were found for fruit quality, all rootstock combinations produced fruit of suitable quality, attending the commercial grading. Our findings evidence the potential of the trifoliate orange-related rootstocks 'C-13' and 'Swingle' to be used as promising rootstocks for 'Shamouti' cultivation in the humid subtropics, promoting genetic diversification and enhancing yield and tree density in new orchards.

Keywords: *Citrus* spp.; scion–rootstock interaction; soil–climate adaptation; tree growth; yield; fruit quality; tree density

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1. Introduction

Citrus spp. has been cultivated for centuries [1], as the fruit has an appreciated flavor and taste. Further, they provide nutrients and vitamin C for human nutrition in addition to being a rich source of antioxidants and phenolic compounds [2]. Four major groups of citrus are economically exploited worldwide: sweet oranges, mandarins, lemons/acid limes, and grapefruits [3]. Sweet oranges (*C.* × *sinensis* (L.) Osb.) comprise the main citrus group, with 75 M tons of fruit harvested in the 2020 season [4]. In 2020, Brazil produced

17 M tons of sweet oranges, followed by India with 9.9 M tons, China with 7.5 M tons, and the United States with 4.8 M tons [4].

Based on the latest survey carried out in the major sweet-orange-producing area of Brazil, the citrus orchards comprise basically the early-season cultivars 'Hamlin' (12%) and 'Valencia Americana' (5%), the mid-season 'Pera Rio' (36%), and the late-season 'Valencia' (25%) and 'Natal' (10%) [5]. Moreover, the sweet orange trees are predominantly grafted on two main rootstocks, the 'Swingle' citrumelo (*C. paradisi* Macf. cv. Duncan × *Poncirus trifoliata* (L.) Raf.) and the 'Rangpur' lime (*C.* × *limonia* (L.) Osb.) [6]. 'Rangpur' has been the most used rootstock in the Brazilian citrus industry for several decades, but 'Swingle' is taking place gradually and becoming the dominant citrus rootstock [6,7]. These rootstocks improve several desirable horticultural traits of the citrus trees.

Trees on 'Rangpur' are precocious, productive, and show tolerance to citrus tristeza virus (CTV), drought, and calcareous soils [8]. Although 'Rangpur' is compatible with most commercial citrus scions and adapted to a wide range of environmental conditions [9], it is susceptible to citrus sudden death (CSD), citrus blight, exocortis, citrus and burrowing nematodes, and *Phytophthora* spp. root rot [8,10]. On the other hand, 'Swingle' has good tolerance to cold, CTV, citrus blight, *Phytophthora* spp., and citrus nematodes [11]. Trees on 'Swingle' grow well in most soils, except in heavy clay or saline soils, poorly drained areas, and under highly calcareous conditions [8,12,13].

A wide range of rootstock introductions are available for citrus production [14–21], but they have not been planted extensively due to citrus growers' preference for traditional varieties. 'C-13' citrange (*C.* × *sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.) rootstock has shown promising performance in previous studies for different sweet orange cultivars, inducing higher yields and excellent fruit quality [14,22–24]. Furthermore, this rootstock shows tolerance to cold, CTV, xyloporosis, and moderate resistance to *Phytophthora* spp. [10,25], but is susceptible to citrus blight and exocortis [26]. 'Cleopatra' (*C. reshni* Hort. ex Tan.) and 'Sunki' (*C. sunki* Hort. ex Tan.) mandarins have been used as rootstocks in several citrus-growing areas including the Brazilian humid subtropical region [8], but at lower proportion compared with 'Swingle' and 'Rangpur' [6]. 'Cleopatra' tolerates high pH, calcareous soils, salinity, and induces good cold and drought tolerance to the citrus trees [27]. Trees have good tolerance to CTV and some viroids, as well as to citrus blight and *Phytophthora* spp. [8,9,27], but are susceptible to nematodes [28]. In addition, trees on this rootstock are vigorous and have low bearing in the first 8–10 years after planting, but produce fruit of high quality though sometimes small [8]. 'Sunki' is reported to possess tolerance to CTV, xyloporosis, citrus blight, and salinity [8]. This rootstock is moderately tolerant to cold and drought but has susceptibility to *Phytophthora* spp. and exocortis [9]. The salinity and drought tolerance induced by 'Sunki' have instigated the interest in replacing 'Rangpur' with 'Sunki' in Brazil [29].

The narrow genetic pool for both scions and rootstocks used in the Brazilian orchards makes the citrus industry vulnerable to biotic and abiotic threats. Orchard diversification by using different scion–rootstock combinations is an efficient measure to promote citrus protection, through increasing the level of genetic diversity [14]. Rootstocks have been widely used for several woody fruit crops such as apple (*Mallus* spp.) [30], pear (*Pyrus* spp.) [31], grape (*Vitis* spp.) [32,33], peach (*Prunus* spp.) [34,35], and others, in order to promote adaptation to biotic and abiotic stress conditions and to enhance horticultural traits. Under this context, the mid-season 'Shamouti' sweet orange (*C.* × *sinensis* (L.) Osb.) is an excellent option for orchard diversification, as the fruits are commercially seedless with adequate soluble solids (SS) contents, low acidity levels, and attractive color, which make them one of the finest fresh sweet oranges [3,13]. 'Shamouti' trees show moderate yields in most citrus-growing regions [3], particularly in the early years of fruit bearing [36,37], though the trees are very productive in some regions [38]. Indeed, fruit yield and quality of 'Shamouti' sweet orange are significantly affected by rootstocks and growing conditions such as climate [3]. Therefore, the evaluation of potential rootstock candidates for this cultivar, under each environmental condition where this sweet orange is intended to be planted, is necessary.

Considering different aspects, the decision to choose a citrus rootstock when establishing a commercial citrus orchard for a specific region should be made on solid bases [12]. The growers must be aware of key traits of each rootstock. For this reason, it is of paramount importance to carry out research regarding the evaluation of different scion–rootstock combinations for a successful recommendation of new citrus plantings. Accordingly, the aim of this study was to assess the horticultural performance of the mid-season 'Shamouti' sweet orange grafted on different rootstocks under a humid subtropical climatic condition in a long-term field experiment.

2. Materials and Methods

2.1. Experimental Location

The experimental orchard was planted in December 2005 at the Londrina Experimental Station of the Instituto de Desenvolvimento Rural do Paraná–IAPAR/Emater (IDR-Paraná) in the municipality of Londrina, Brazil (23°21′34″ S, 51°09′53″ W, and altitude of 585 m). The climate of the region is humid subtropical (Cfa) according to the Köppen–Geiger climate classification [39], with annual maximum and minimum air temperatures of 27.3 °C and 16.1 °C, respectively, annual average rainfall of 1641 mm (Figure S1), and relative humidity of 70.5% [40]. The soil is a dystropheric red oxisoil with a clay texture, and slightly wavy to flat relief [41]. The chemical composition of the soil at 0–60 cm depth is described in Table S1.

2.2. Plant Material

Scion and rootstock propagative materials were provided by the Active Germoplasm Bank of Citrus (AGB–Citrus) of the IDR-Paraná established in Londrina, state of Paraná, Brazil. Five rootstocks were included in this study, 'Rangpur', 'Cleopatra', 'Sunki', 'Swingle', and 'C-13'. The 'Shamouti' sweet orange I-184 accession was used as scion. The nursery trees were planted at spacing of 7.0 m $\times$ 4.0 m between- and in-rows, respectively, with a total of 357 trees·ha^{-1}. The experimental design was a randomized block with five treatments (rootstocks), six replicates, and two trees per plot.

2.3. Tree Management

Tree management was based on the standard commercial recommendations for sweet orange cultivation in the state of Paraná, including conventional pest and disease management programs [26,42]. Disease and insect pest management involved preventive copper spraying for citrus canker (*Xanthomonas citri* subsp. *citri*) control during the entire experimental period, and fortnight foliar spraying of insecticides for the Asian citrus psyllid (ACP; *Diaphorina citri* Kuwayama) control from 2014 through 2017, when the vector of huanglongbing (HLB) was present in the experimental location. Fertilization was performed in response to soil chemical analyses (Table S1). Fertilizers were applied four times a year from August to March to supply the maintenance requirements for nitrogen (N), potassium (K), phosphorus (P), boron (B), and zinc (Zn) for the entire duration of the experiment. The doses applied of each fertilizer were based on tree age [26]. Weeds were managed with periodic mowing (3 times a year) using an ecological mower between-rows and herbicide sprays in-rows (glyphosate) when required, according to the commercial recommendation. Trees were not irrigated and relied on natural rainfall.

2.4. Tree Size Measurements

Tree growth measurements were carried out in September from 2007 through 2017, just after the annual harvests. The vegetative growth for each scion–rootstock combination was determined based on the 2016–2017 data, while the growth rate was calculated based on the annual data from 2007 through 2017, considering tree height and canopy volume.

Tree height and canopy diameter were used to calculate canopy volume as previously described by Mendel [43]:

$$CV = \frac{2}{3} \times \pi \times CR^2 \times TH, \quad (1)$$

where CV = canopy volume (m^3); CR = canopy radius (m); and TH = tree height (m).

In addition, the trunk circumference was measured with a cloth measuring tape 10 cm above and below the graft union, and then converted to diameter. The trunk diameter index was calculated based on the ratio between trunk diameter above (scion) and below (rootstock) the graft union.

2.5. Fruit Yield Assessment

Annual yields were determined in July from 2009 up to 2017, comprising nine harvest seasons. Cumulative yields were comprised after the annual harvests. Yield efficiency was calculated based on the relationship between fruit yield (kg·tree^{-1}) and canopy volume (m^3) determined in the 2016–2017 period. The results were expressed in kg·m^{-3}. The alternate bearing index was also determined according to Pearce and Doberšek-Urbanc [44]:

$$ABI = \frac{1}{n-1} \times \left\{ \frac{|a_2 - a_1|}{a_2 + a_1} + \frac{|a_3 - a_2|}{a_3 + a_2} + \ldots + \frac{|a_n - a_{n-1}|}{a_n + a_{n-1}} \right\}, \quad (2)$$

where ABI = alternate bearing index, n = number of years, and a_1, a_2, … , $a_{(n-1)}$, $a_{(n)}$ = yields of the corresponding years.

2.6. Fruit and Juice Quality Evaluation

Fruit and juice quality were determined based on 10-fruit samples per plot. Samples were randomly collected at 1–2 m tree height of the two innermost trees per block, in June from 2009 to 2017, before annual harvests. The evaluated parameters were based on the averages for the evaluation period. Fruit length and diameter were measured with a digital Vernier caliper (ABS, Mitutoyo, Kawasaki, Japan). Fruits were weighed and classified according to the fresh citrus and industrial standards [45,46]. The fruit shape index was calculated based on the relationship between fruit length and diameter. Fruit samples were juiced using an extractor (Croydon, Duque de Caxias, Brazil). Juice content was determined based on the relationship between juice weight and fruit weight:

$$JC = \frac{JW}{FW} \times 100, \quad (3)$$

where JC = juice content (%); JW = juice weight (g); and FW = fruit weight (g).

The soluble solids (SS) content was measured with a digital refractometer (PAL-3, Atago Co., Ltd., Tokyo, Japan) in an aliquot (0.3 mL) of undiluted juice. The temperature was corrected to 20 °C, and the results were expressed in °Brix. Titratable acidity (TA) was determined by titration of 0.1 N NaOH in 25 mL of juice, using an automatic titrator (TitroLine® easy, Schott Instruments GmbH, Mainz, Germany) and phenolphthalein as a visual end-point indicator. Acidy level was expressed in grams of citric acid per 100 mL of juice (g·100 mL^{-1}) [47]. The ratio between SS content and TA (SS/TA) was also calculated. The technological index, which indicates the amount of SS content per standard citrus box (total capacity of 40.8 kg), was calculated according to the method proposed by Di Giorgi et al. [48]:

$$TI = \frac{SS \times JC \times 40.8}{10,000}, \quad (4)$$

where TI = technological index (kg TSS·box^{-1}); SS = soluble solids (°Brix); and JC = juice content (%).

2.7. Plant Density and Yield Estimates for New Plantings

The number of trees per hectare (tree density) and tree and row spacing were estimated for the 'Shamouti' trees grafted on the tested rootstocks. The estimates study assumed a free spacing of 2.5 m between-rows (canopy diameter + 2.5 m), for better equipment movement within the orchard, and 15% tree overlap in-rows (canopy diameter × 0.85) [49]. Fruit yield was estimated according to the theoretical number of trees per hectare and the average of fruit yield per tree determined for 2009–2017. The SS yield was determined according to the estimated yield and expressed in tons of SS per hectare (t SS·ha^{-1}):

$$\text{SS Yield} = \frac{\text{SS} \times \text{JC} \times \text{YE}}{10{,}000}, \quad (5)$$

where SS Yield = soluble solids yield (t SS·ha^{-1}); SS = soluble solids (°Brix); JC = juice content (%); and YE = yield estimation. Adapted from Di Giorgi et al. [48].

2.8. Statistical Assessment

The data were analyzed according to the experimental design and tested for normal distribution and homogeneity at $p \leq 0.05$. All data were submitted to ANOVA followed by the comparison of means using the Tukey's post hoc test at $p \leq 0.05$. Additionally, tree height and canopy volume means were adjusted to the polynomial regression model ($p \leq 0.05$) in order to elucidate the growth pattern of 'Shamouti' trees on multiple rootstocks over 11 years (2007–2017). Significant variables were taken together and submitted to the multivariate analysis using a mean value for each parameter and rootstock, in which a principal component analysis (PCA) was built. All data were processed in R v. 4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis and graphics composition.

3. Results

3.1. Tree Size Measurements

Tree growth measurements were assessed in 2016–2017 when the 'Shamouti' trees were 11 to 12 years old. The rootstocks significatively ($p \leq 0.05$) affected tree size (Table 1). Trees on the 'Sunki' rootstock were the tallest ones at 4.6 m, while the trees on 'Swingle' and 'C-13' were the smallest, at less than 4.0 m in height (Figure 1). Tress on 'Rangpur' and 'Cleopatra' had intermediate heights, all above 4.0 m. 'Swingle' also induced the smallest canopy diameter and volume, at 4.6 m and 43 m^3, respectively, significatively different from the ones on 'Sunki', which were the largest at 5.1 m and 64 m^3, respectively. No difference was observed among the rootstocks for trunk diameters, which ranged between 24.9 and 27.1 cm. However, the scion trunk diameters were significantly ($p \leq 0.001$) different, with the trees on 'Swingle' and 'C-13' rootstocks with larger scion trunk diameters than the ones on 'Rangpur', 'Cleopatra', and 'Sunki'. These differences clearly affected the trunk diameter index, which ranged from the lowest at 0.53 for the ones on 'Swingle' up to 0.79 for those on 'Sunki'. The power of growth development was also variable (Figure 2). The 'Shamouti' trees grafted on 'Swingle' and 'C-13', the least vigorous rootstocks, ceased height growth by 8 years old (indicated by the red arrow in the Figure 2), while the ones on the other rootstocks stopped growth by the age of ~10 years, the most vigorous ones. This pattern was also observed for the canopy volume growth (Figure 2), in which trees on 'Swingle' and 'C-13' rootstocks grew faster in the early stage of tree development rather than the ones on 'Sunki', 'Cleopatra', and 'Rangpur'.

Table 1. Tree size of 'Shamouti' sweet orange grafted on different rootstocks grown in Londrina, state of Paraná, Brazil. Means were based on the 2016–2017 seasons.

Rootstock	Tree Height (m)	Canopy Diameter (m)	Canopy Volume (m^3)	Rootstock Trunk Diameter [1] (cm)	Scion Trunk Diameter [1] (cm)	Trunk Diameter Index [2]
Rangpur	4.24 ± 0.10 b [3]	5.04 ± 0.17 ab	56.5 ± 4.38 abc	24.9 ± 1.32	19.3 ± 0.69 a	0.776 ± 0.05 a
Cleopatra	4.26 ± 0.13 ab	5.08 ± 0.12 ab	57.7 ± 4.06 ab	25.3 ± 0.69	19.5 ± 0.59 a	0.770 ± 0.02 a
Sunki	4.63 ± 0.25 a	5.12 ± 0.24 a	64.1 ± 9.40 a	25.9 ± 1.81	20.5 ± 1.19 a	0.792 ± 0.02 a
Swingle	3.88 ± 0.21 b	4.60 ± 0.27 b	43.4 ± 6.59 c	27.1 ± 2.18	14.3 ± 0.97 b	0.527 ± 0.01 c
C-13	3.91 ± 0.33 b	4.85 ± 0.50 ab	49.3 ± 12.9 bc	25.8 ± 2.16	15.2 ± 1.47 b	0.589 ± 0.02 b
CV (%)	5.39	5.80	15.17	6.83	5.87	4.16
F value	10.9 ***	3.34 *	5.71 **	1.31 ns	43.6 ***	110.9 ***

[1] Trunk diameters were based on trunk circumference measurements, 10 cm above and below the graft union. [2] Trunk diameter index was expressed as the ratio between scion and rootstock trunk diameters. [3] Means followed by the same letter in the column do not differ significantly according to Tukey's post hoc test ($p \leq 0.05$). Significance level: ns, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Figure 1. Nine-year-old 'Shamouti' sweet orange trees grafted on 'C-13' rootstock grown in Londrina, state of Paraná, Brazil.

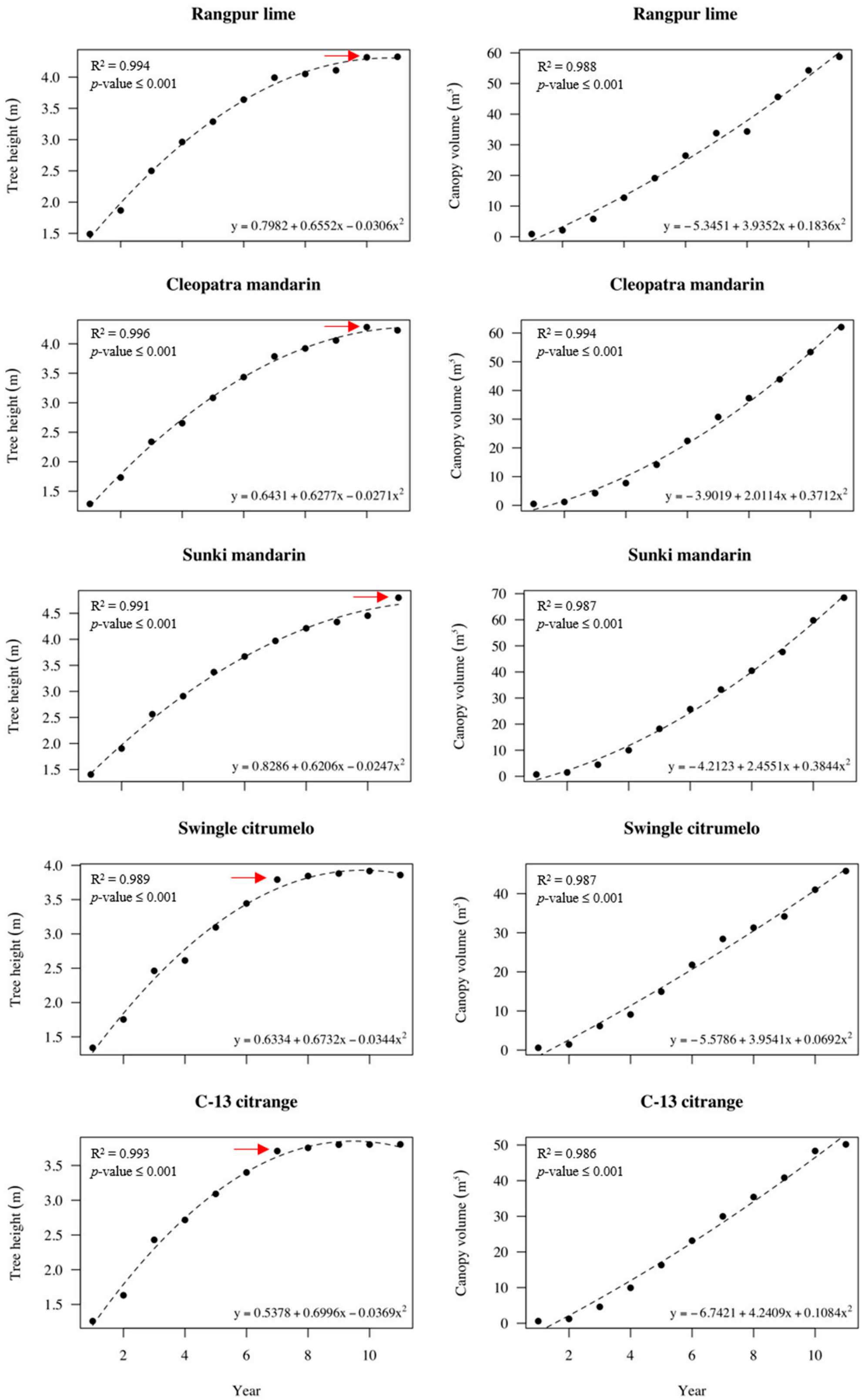

Figure 2. Tree height and canopy volume of 'Shamouti' sweet orange trees grafted on different rootstocks under the humid subtropical climate, from the period of two (2007) up to twelve (2017) years of age. Red arrows indicate the time when trees ceased growth in height.

3.2. Fruit Yield Assessment

Differences ($p \leq 0.05$) were observed among the rootstock in relation to the yield performance of the 'Shamouti' trees (Table 2). Although fruit yield was low in the first three seasons (Table 2), a tendency for early bearing was observed in the trees grafted on the 'Swingle' rootstock, with ~50 kg of fruit per tree in the 2010 season. After 2012, trees grafted on most of the rootstocks had regular yields, ranging from 47 kg up to 132 kg per tree, depending on the cropping season and the rootstock. On the other hand, trees grafted on 'Cleopatra' did not show reasonable yield performance during the evaluated period, from 2009 up to 2017, reaching a cumulative yield of only 255 kg per tree. In contrast, the other rootstocks induced a highly significant ($p \leq 0.001$) cumulative yield for the trees, which ranged from 463 kg on 'Sunki' up to 549 kg per tree on 'Swingle'. Further, trees on 'Swingle' also had the best yield efficiency, with 2.03 kg of fruit per cubic meter of tree canopy. This yield efficiency was significantly higher ($p \leq 0.001$) than those observed for the trees on the mandarin rootstocks, with 0.65 kg·m^3 for 'Cleopatra' and 1.23 kg·m^3 for 'Sunki'. Moreover, these mandarin rootstocks imparted the highest alternate bearing indices (0.556 and 0.456) for the 'Shamouti' trees, higher than those observed for trees on 'Rangpur', 'Swingle', and 'C-13' rootstocks.

Table 2. Annual yield, cumulative yield, yield efficiency, and alternate bearing index of 'Shamouti' sweet orange trees grafted on different rootstocks grown in Londrina, state of Paraná, Brazil, from 2009 up to 2017.

	Rootstocks					CV (%)	*F* Value
	Rangpur	Cleopatra	Sunki	Swingle	C-13		
Annual yield (kg·tree^{-1})							
2009	1.04 ± 1.9 ab [2]	0.56 ± 0.2 b	2.25 ± 1.3 ab	5.24 ± 5.4 a	1.90 ± 0.9 ab	119.0	2.92 *
2010	11.8 ± 7.8 bc	7.23 ± 6.2 c	16.7 ± 10.0 bc	49.0 ± 20.7 a	27.5 ± 13.0 b	49.1	13.7 ***
2011	21.9 ± 9.1 a	1.05 ± 0.8 c	3.51 ± 2.1 bc	9.43 ± 5.2 bc	13.4 ± 12.1 ab	70.7	8.50 ***
2012	28.1 ± 7.3 bc	11.2 ± 7.8 c	28.1 ± 8.4 bc	64.5 ± 10.0 a	45.2 ± 18.0 b	30.8	20.5 ***
2013	77.0 ± 14.8 a	37.1 ± 12.1 b	66.0 ± 20.4 ab	66.5 ± 25.3 ab	77.1 ± 21.9 a	28.4	4.74 **
2014	126.1 ± 12.5	95.3 ± 6.3	131.9 ± 14.1	116.4 ± 45.3	117.9 ± 22.4	20.5	2.01 ns
2015	48.1 ± 21.0 ab	28.1 ± 12.0 b	61.6 ± 15.2 a	62.4 ± 13.0 a	46.6 ± 11.1 ab	30.4	5.19 **
2016	81.9 ± 13.1 a	46.2 ± 8.5 b	81.3 ± 10.1 a	77.6 ± 17.4 a	73.5 ± 17.2 a	19.2	6.90 **
2017	71.8 ± 48.0 ab	28.6 ± 27.1 b	71.4 ± 37.9 ab	98.3 ± 28.5 a	78.3 ± 31.7 ab	54.2	2.72 *
Cumulative yield (kg)	467.7 ± 71.7 a	255.2 ± 36.1 b	462.7 ± 52.6 a	549.3 ± 112.7 a	481.4 ± 43.7 a	16.1	14.3 ***
Yield efficiency (kg·m^{-3}) [1]	1.35 ± 0.5 abc	0.65 ± 0.3 c	1.23 ± 0.3 bc	2.03 ± 0.4 a	1.58 ± 0.4 ab	22.1	8.44 ***
Alternate bearing index	0.399 ± 0.07 b	0.556 ± 0.08 a	0.456 ± 0.04 a	0.402 ± 0.03 b	0.414 ± 0.07 b	14.4	6.32 **

[1] Yield efficiency was based on the average of fruit yield and canopy volume in 2016 and 2017. [2] Means followed by the same letter in the row do not differ significantly according to Tukey's post hoc test ($p \leq 0.05$). Significance level: ns, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

3.3. Fruit and Juice Quality Evaluation

The quality of the fruit of 'Shamouti' was assessed from 2009 up to 2017, based on the results of these nine seasons (Table 3). Further, significant differences ($p \leq 0.05$) were observed for all physical attributes of the fruit produced by the trees on the different rootstocks (Table 3). Trees on 'Swingle' and 'C-13' rootstocks produced larger fruit than the ones on 'Rangpur', 'Cleopatra', and 'Sunki'. Means of fruit length were above 83 mm from trees on 'Swingle' and 'C-13', but below 80 mm for all from the ones on the other rootstocks. Fruit diameters of all 'Shamouti' were between 72 and 78 mm. All fruit had a shape index of 1.0, indicating a round shape, typical of sweet oranges. The weight was also variable for fruit from the trees on the different rootstocks, though the trees on 'Swingle' and 'C-13' produced the heaviest fruit, 259 and 256 g, respectively. They were heavier than those from trees on 'Cleopatra', 'Rangpur', and 'Sunki', at 205, 208, and 220 g, respectively. The number of seeds per fruit ranged from six to eight for fruits from trees on 'Cleopatra', 'Sunki', and 'C-13' rootstocks.

Table 3. Nine-season average fruit quality of 'Shamouti' sweet orange trees grafted on different rootstocks grown in Londrina, state of Paraná, Brazil, from 2009 up to 2017.

Rootstock	Fruit Length FL (mm)	Fruit Diameter FD (mm)	Fruit Shape (FL/FD)	Fruit Weight (g)	Number of Seeds
Rangpur	78.4 ± 2.2 b [1]	72.6 ± 1.6 b	1.079 ± 0.01 ab	208 ± 10.5 bc	7 ± 0.6 ab
Cleopatra	77.7 ± 0.9 b	72.2 ± 0.9 b	1.075 ± 0.01 b	205 ± 5.0 c	6 ± 0.6 b
Sunki	80.0 ± 0.9 b	73.2 ± 0.6 b	1.092 ± 0.01 a	220 ± 4.5 b	8 ± 0.9 a
Swingle	83.8 ± 1.6 a	77.8 ± 1.0 a	1.077 ± 0.01 ab	259 ± 11.0 a	7 ± 0.8 ab
C-13	84.4 ± 0.8 a	76 9 ± 0.9 a	1.097 ± 0.01 a	256 ± 7.8 a	6 ± 0.7 b
CV (%)	1.83	1.33	1.10	3.11	11.6
F value	26.3 ***	41.9 ***	3.89 *	78.9 ***	6.02 **

[1] Means followed by the same letter in the column do not differ significantly according to Tukey's post hoc test ($p \leq 0.05$). Significance level: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Rootstocks also affected the juice content of the 'Shamouti' fruit (Table 4). Trees on 'Cleopatra' produced fruit with the highest juice content, 47.8%, while those from trees on 'C-13' had the lowest content, 45.1%. Further, fruit of the trees on the other rootstocks had juice content above 47%. In contrast, no differences were observed in relation to soluble solids (SS) content and technological index among the fruit produced by trees grafted on the different rootstocks. Furthermore, the SS content ranged between 9.42 and 9.59 °Brix, while the technological index varied from 1.75 to 1.84 kg SS·box^{-1}. In contrast, significant differences were found for the titratable acidity (TA), as the acidity level went from 1.02 for fruit of the trees on 'C-13' up to 1.13 for the ones from trees on 'Sunki', based on a nine-season average. The acidity level played a major role on the SS/TA ratio, as the fruit produced by the trees on 'Sunki' had the lowest ratio, the one that peaked higher for juice acidity, compared with those of the fruit from trees on 'C-13', 'Cleopatra', and 'Rangpur' rootstocks.

Table 4. Nine-season average juice quality of 'Shamouti' sweet orange fruit from trees grafted on different rootstocks grown in Londrina, state of Paraná, Brazil, from 2009 to 2017.

Rootstock	Juice Content (%)	Soluble Solids SS (°Brix)	Titratable Acidity TA (g 100·mL^{-1})	Ratio (SS/TA)	Technological Index (kg SS·box^{-1})
Rangpur	46.0 ± 0.8 bc [1]	9.58 ± 0.4	1.06 ± 0.03 bc	9.27 ± 0.2 a	1.80 ± 0.06
Cleopatra	47.8 ± 0.3 a	9.42 ± 0.1	1.03 ± 0.01 bc	9.33 ± 0.1 a	1.78 ± 0.03
Sunki	47.1 ± 0.6 ab	9.59 ± 0.1	1.13 ± 0.02 a	8.69 ± 0.2 b	1.84 ± 0.05
Swingle	47.1 ± 0.6 ab	9.49 ± 0.1	1.07 ± 0.03 b	9.10 ± 0.2 ab	1.83 ± 0.05
C-13	45.1 ± 0.8 c	9.48 ± 0.5	1.02 ± 0.03 c	9.55 ± 0.4 a	1.75 ± 0.12
CV (%)	1.49	3.11	2.31	2.99	3.36
F value	14.3 ***	0.37 ns	19.0 ***	8.28 ***	2.48 ns

[1] Means followed by the same letter in the column do not differ significantly according to Tukey's post hoc test ($p \leq 0.05$). Significance level: ns, non-significant; ***, $p \leq 0.001$.

3.4. Plant Density and Yield Estimates for New Plantings

The estimates for new plantings of 'Shamouti' trees grafted on the different rootstocks were also determined, including orchard arrangement, tree density, and yield (Table 5). Trees on 'Sunki' required the largest spacing within rows and trees, 7.6 and 4.2 m, respectively, resulting in the lowest tree density, 303 trees·ha^{-1}. In contrast, trees on 'Swingle' and 'C-13' provided the highest tree density, 363 and 337 trees·ha^{-1}, respectively, due to their short row spacing (7.1 and 7.3 m, respectively) and tree spacing (3.9 and 4.1 m, respectively). The estimates for the trees on 'Rangpur' and 'Cleopatra' were intermediate in regard to row and tree spacing and tree density. In regard to the estimates of fruit yield, most tested rootstocks induced high yields for the 'Shamouti' trees, from 23.0 up to 28.4 t·ha^{-1}, except for 'Cleopatra'. The estimated yields also had a direct relation with the soluble solids (SS) yields, ranging from 1.015 up to 1.270 t SS·ha^{-1}, while for the trees on 'Cleopatra' was only 0.545 t SS·ha^{-1}.

Table 5. Estimates [1] of minimum row and tree spacing, maximum tree density, fruit yield, and soluble solids (SS) yield for new plantings of 'Shamouti' sweet orange trees grafted on different rootstocks. Means were based on the scion–rootstock performance under the humid subtropical climate, in the north of the state of Paraná, Brazil, from 2005 to 2017.

Rootstock	Row Spacing (m)	Tree Spacing (m)	Tree Density (Trees·ha^{-1})	Estimate Yield (t·ha^{-1})	SS Yield (t SS·ha^{-1})
Rangpur	7.54 ± 0.1 ab [2]	4.28 ± 0.1 ab	311 ± 18.6 ab	23.0 ± 5.6 a	1.015 ± 0.2 a
Cleopatra	7.58 ± 0.1 ab	4.31 ± 0.1 ab	307 ± 12.4 ab	12. 1 ± 3.5 b	0.545 ± 0.1 b
Sunki	7.62 ± 0.2 a	4.35 ± 0.2 a	303 ± 22.4 b	23.0 ± 4.9 a	1.041 ± 0.2 a
Swingle	7.10 ± 0.2 b	3.91 ± 0.2 b	363 ± 28.5 a	28.4 ± 4.6 a	1.270 ± 0.2 a
C-13	7.35 ± 0.5 ab	4.12 ± 0.4 ab	337 ± 60.7 ab	24.1 ± 3.5 a	1.030 ± 0.1 a
CV (%)	3.85	5.80	10.7	20.9	21.5
F value	3.34 *	3.34 *	3.22 *	10.1 ***	9.40 ***

[1] Estimates study was based on vegetative, yield, and fruit quality data of 'Shamouti' sweet orange trees grafted on different rootstocks; tree density and row/tree spacing projections were calculated according to De Negri and Blasco [49] and used to estimate fruit yield and SS yield. [2] Means followed by the same letter in the column do not differ significantly according to Tukey's post hoc test ($p \leq 0.05$). Significance level: *, $p \leq 0.05$; ***, $p \leq 0.001$.

3.5. Multivariate Analysis

A principal component analysis (PCA) was performed based on multiple horticultural parameters involving tree size, yield, fruit quality, and planting density and yield estimations for the 'Shamouti' trees grafted on different rootstocks (Figure 3). The first two principal components identified 81.6% of the data variance, as the Dim1 explained 59.0% and the Dim2 explained 22.6%. The studied individuals (rootstocks) were segregated in three distinct regions (clusters: Col.1, Col.2, and Col.3) in a multidimensional space, grouping according with their similarities to the evaluated parameters (Figure 3). The first cluster was formed by 'Sunki' and 'Rangpur' (Col.1). These rootstocks were recognized to induce vigorous growth and moderate yield performance for the 'Shamouti' trees, but with lower tree density estimation. Additionally, fruit produced by trees on these rootstocks had the highest number of seeds and high TA and SS contents. Similarly, 'Cleopatra' was characterized by inducing vigorous growth to the scion, but with poor yield performance, and it segregated from all other rootstocks (Col.2). 'Swingle' and 'C-13' belonged to the same group, as they show similarities to several parameters (Col. 3). They were characterized by imparting moderate growth and higher yields to 'Shamouti' trees, in addition to promoting better physical fruit quality and higher tree density in comparison with all other rootstock genotypes tested.

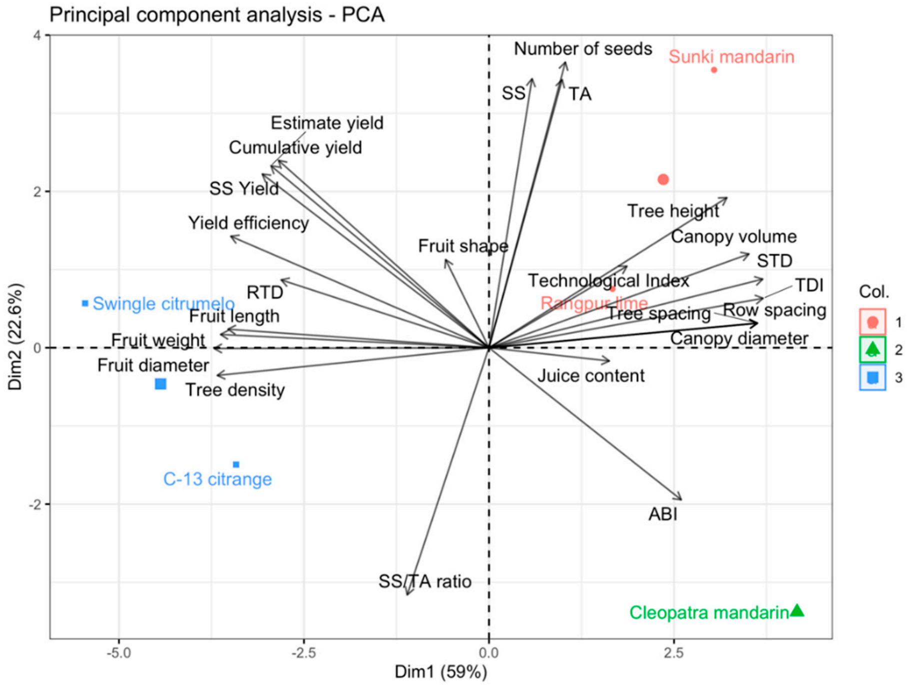

Figure 3. Principal component analysis (PCA) for vegetative growth, yield, and fruit quality variables of 'Shamouti' sweet orange trees grafted on different rootstocks. The variables were arranged according to their principal component scores and the individuals (rootstocks) were grouped into three distinct clusters (Col.): 1, 2, and 3. Variables: tree height (m); canopy volume (m); canopy diameter (m); RTD—rootstock trunk diameter (cm); STD—scion trunk diameter (cm); TDI—trunk diameter index (STD·RTD^{-1}); cumulative yield (kg·tree^{-1}); yield efficiency (kg·m^{-3}); ABI—alternate bearing index; SS—soluble solids (°Brix); TA—titratable acidity (g·100 mL^{-1}); SS/TA ratio (SS·TA^{-1}); juice content (%); TI—technological index (kg SS·box^{-1}); fruit weight (g); fruit length (mm); fruit diameter (mm); fruit shape; and number of seeds per fruit.

4. Discussion

Rootstock plays an important role in citrus production, as it may regulate several horticultural traits of the tree, in addition to the resistance or tolerance to biotic and abiotic stresses [8,12,50]. In our study, some of these traits were significantly influenced by the tested rootstocks for 'Shamouti' sweet orange. Therefore, the evaluation of different scion–rootstock combinations is of paramount importance for the establishment of new citrus plantings under a specific soil–climate condition, as it may contribute to orchard diversification and crop protection.

In regard to tree size, the 'Sunki' rootstock induced the most vigorous growth for the 'Shamouti' trees under the humid subtropical climate of the north region of the state of Paraná, Brazil (Table 1; Figure 3). Trees on this rootstock had the highest height and canopy volume, 4.6 m and 64 m^3, respectively, at 11–12 years old. These values were close to those of the ones on 'Cleopatra' and 'Rangpur' (Table 1), which were also confirmed by the principal component analysis (Figure 3). On the other hand, trees on 'Swingle' and 'C-13' were established early in the field (Figure 1), but still had the lowest vigor, displaying heights of 3.9 m and canopy volume between 43 and 49 m^3 (Figure 3). These values are close to those reported in another study for seven-year-old 'Shamouti' grafted on 'Rangpur' [37]. Furthermore, Hodgson [51] and Albrigo et al. [13] described 'Shamouti' as very vigorous with upright growth; however, it may show a decrease in tree size when grafted on some rootstocks as 'Swingle'.

Tree density and horticultural practices are clearly dependent on tree vigor. Scion–rootstock combinations that result in low to medium vigor and higher-yield trees have been highly demanded by the citrus growers. These combinations may guarantee higher tree density in the orchards and facilitate field operations such as spraying, harvesting, pruning, and equipment movement within the orchards [13]. Furthermore, only the less vigorous rootstocks would allow the feasibility of mechanical harvesting [20]. In our study, the 'Swingle' rootstock maximized the tree density of 'Shamouti' trees, as shown in the study of the estimates for new plantings (Table 5; Figure 3). This rootstock allowed 363 trees per hectare in new plantings, higher than that estimated for the 'Sunki' at 303 trees·ha^{-1}.

Due to the vigorous growth, the number of 'Shamouti' trees per hectare is relatively low compared to the number of trees currently planted for most of the sweet orange cultivars in the Brazilian citrus orchards, 581 trees per hectare [5]. However, the rootstock can be crucial in enhancing tree density and yield for 'Shamouti', as observed for 'Swingle' and 'C-13', which provided an increase of 17% and 8% in the number of trees per hectare and 13% and 3% of yield, respectively, in comparison with 'Rangpur' (Table 5). Therefore, the selection of proper planting density and orchard arrangement certainly improve yield, fruit quality, and field operations [13]. This is particularly important in the citrus areas where HLB is endemic, the most devastating citrus disease that has reduced the lifespan of the orchards. Further, higher planting densities favor the reduction in the detrimental effects caused by the massive elimination of the HLB-symptomatic trees [52–55]. Attention should be given to tree density in regard to meeting the needs of the citrus growers, as the use of a citrus rootstock that induces smaller tree size could be very advantageous for 'Shamouti', promoting higher yields (Figure 3). This procedure maximizes yield efficiency in high-density plantings, as in the case of 'Swingle' and 'C-13'. Therefore, growers must adjust tree density in the orchards according to the tree morphology, yield, and vigor of the desired scion–rootstock combination.

Scion–rootstock compatibility is another important characteristic to be considered in citrus selection, as it regulates yield, fruit quality [18], and tree longevity [56]. Graft incompatibility can be apparent between citrus rootstock and scion and may occur in the early stages of perennial woody plants [57]. However, in some cases they may become apparent only several years after grafting [58]. The incompatibility between plant tissues is a complex response triggered by biochemical, physiological, and anatomical interactions that may result in an overgrowth of the scion or the rootstock [59,60]. This incompatibility limits the uptake and transport of water and nutrients and hormone production and transport, as well as the large-scale movement of proteins and RNAs, which curbs the normal development and yield of the grafted trees [56,61]. The ratio between scion and rootstock trunk diameters is usually used as an indicator of graft compatibility, in which a quotient of 1.0 indicates a full compatibility between scion and rootstock [16,62,63]. However, the difference in trunk diameter may be related to the metabolism and genetic traits of the grafted tree, rather than the true incompatibility between the scion–rootstock combinations [8,64]. Therefore, the scion–rootstock compatibility must be based on the overall horticultural performance of the grafted tree and not solely on this morpho-anatomical differences.

The trunk diameter ratio observed in our study was higher for the combinations between 'Shamouti' and the rootstocks 'Sunki', 'Cleopatra', and 'Rangpur'. In contrast, low ratios were recorded for the 'Swingle' and 'C-13' combinations. Our results are close to those reported by Georgiou and Gregoriou [38] evaluating 14 rootstocks for 'Shamouti' trees in Cyprus. These authors recorded a lower diameter ratio for 'Swingle' (0.76) than 'Rangpur' (0.85) after nine years of planting. Regardless of the marked differences in growth rate and vigor between scion and rootstock trunk diameters, the 'Swingle' and 'C-13' combination did not show any deleterious effects in the grafted trees during the first 12 years of planting. These *P. trifoliate* related rootstocks are recognized to develop an overgrowth below graft union for most commercial citrus cultivars because of their rapid growth rate, without showing any hazard for trunk strength or tree physiology [8,65]. Similar overgrowth has been reported in previous studies for different citrus scions grafted on citrumelo and citrange rootstocks [14,24,38,66–68].

Grafted citrus trees usually start bearing fruits at 2 to 3 years after planting, though they may reach full production by 5 to 6 years of age [50]. Scion–rootstock combinations that yield earlier are very advantageous, particularly under the current endemic HLB condition. This tendency of early cropping has been observed in our study for the trees grafted on 'Swingle' and 'C-13', with the highest cumulative yields after 12 years of planting (2005), among the rootstocks included in the study (Table 2). Similarly, 'Swingle' citrumelo and some citrange rootstocks such as 'Carrizo', 'Yuma', and 'Morton' also imparted higher yields to 'Shamouti' trees grown in the Mediterranean region [38]. Moreover, trees on 'Swingle' and 'C-13' have also been reported as being very productive for some other citrus scions, including 'Cadenera' and 'Salustiana' sweet oranges [14,21] and 'Montenegrina' and 'Emperor' mandarins [67,68] grown under the soil–climate conditions of northern Paraná, Brazil.

In contrast, 'Shamouti' trees grafted on 'Cleopatra' had poor yield for the entire duration of our study, with the lowest yields. This low yield performance of the trees on 'Cleopatra' is related to its limited yield efficiency in the early stages of the tree growth and development (Table 2), combined with its vigorous vegetative growth (Table 1; Figure 2). Most of its energy-storing monosaccharide photosynthates were likely directed to vegetative growth at the expenses of fruit development and growth, as reported in previous studies [50,69]. Trees on this rootstock produce a reduced number of fruits in the early years of bearing [17], typical of its slow growth tendency in the early stages of tree development (Figure 2). 'Cleopatra' also showed low bearing tendency in previous studies involving some citrus scions such as 'Valencia', 'Cadenera', and 'Salustiana' sweet oranges [14,21,27], 'Navelina' and 'Washington' navel oranges [24,70], 'Marisol' clementine [16], 'Montenegrina' mandarin [67], and 'Allen Eureka', 'Fino', and 'Lapithkiotiki' lemons [15,71,72].

In addition to the yield potential, citrus scion–rootstock combinations should produce fruit of suitable quality in order to attend the consumer's requirements [45,46,73]. However, fruit quality in citrus is governed by a multiple-complex interaction between rootstock and scion cultivars due to hormone balance, biotic and abiotic factors, and horticultural practices [12,71,74]. In our study, significant differences were observed among the rootstocks in regard to quality of the 'Shamouti' fruit (Tables 3 and 4). Fruits produced by trees on 'C-13' and 'Swingle' were larger and heavier than those produced by trees on all other rootstocks. Despite this variation, trees grafted on all five rootstocks yielded fruit of large size ($\geq$71 mm), obtaining an excellent grade (A) based on the Brazilian fresh citrus standards [45]. Furthermore, the diameter of the 'Shamouti' fruit was above the minimum established for the international fresh fruit market, 53 mm [73]. The fruit shape was round, as the indices ranged between 1.07 and 1.09, similar to those reported by Paula et al. [37], typical of this sweet orange cultivar [51]. The number of seeds per fruit of 'Shamouti' was low, between six and eight, similar to the amount reported for 'Shamouti Masry' fruit, eight seeds per fruit [75]. Although the trees of all scion–rootstock combinations produced seedy fruit, most of them could be still considered commercially as seedless, according to Albrigo et al. [13].

The juice content of the 'Shamouti' fruit was high, ranging from 45.1 up to 47.8% (Table 4). 'Cleopatra' conferred the highest juice content to the fruit, while 'C-13' imparted the lowest juice concentration. 'Cleopatra' also induced higher juice content to the fruit of other citrus cultivars including 'Salustiana' [14], 'Lane Late', and 'Delta' sweet oranges [18] and 'Emperor' mandarin [68]. The highest juice content in fruit produced by citrus trees grafted on this rootstock may be related to the lower yield potential (Table 2), as less productive trees usually produce fruit of better quality in comparison with more productive ones due to the competition for water, nutrients, and photosynthates [13]. In general, fruit of 'Shamouti' trees grafted on all rootstocks attained the minimum juice content established by the fresh market and the processing industry [45,73,76]. No such variations were found among the rootstock treatments for the soluble solids (SS) content. It ranged from 9.42 up to 9.59 °Brix (Table 4). This range is close to that reported for the same scion grafted on several rootstocks [38], including 'Rangpur' [37], under the Mediterranean and the humid subtropical regions, respectively.

On the other hand, the acidity level of the juice was variable within the different scion–rootstock combinations. The highest titratable acidity (TA), expressed as grams of citric acid per 100 mL of juice (g·100 mL^{-1}), was for the fruit produced by the trees grafted on the 'Sunki' rootstock, 1.13 g·100 mL^{-1}, while the lowest concentration was for the fruit from trees on 'C-13', 1.02 g·100 mL^{-1}. Similar acidity levels were reported by Georgiou and Gregoriou [38] for 'Shamouti' fruit, ranging between 0.96 and 1.15 g·100 mL^{-1}, depending on the rootstock. In general, the acidity level recorded for the 'Shamouti' fruit in our study is within the range demanded by the fresh market and the juice processing industry, 0.7 to 1.2 g·100 mL^{-1} [46,77]. In regard to the maturity index (SS/TA ratio) of the 'Shamouti' sweet orange juices, the fruit of the trees on 'Sunki' showed the lowest ratio, while the ones of the trees on 'C-13' had the highest index, 9.55. The SS/TA ratios were above the threshold established by the international standards for the fresh market, 6.5:1 [73], and close to those of a previous study for the same sweet orange cultivar [38]. However, these values were far below the minimum demanded by the juice processing industry, which is 12 [77], reinforcing the destination of this sweet orange to the fresh fruit market. The technological index (TI) for the 'Shamouti' fruit did not show significant differences, ranging between 1.75 and 1.84 kg SS·box^{-1}. These values are similar to those reported for the fruit of 'Shamouti' trees grafted on 'Rangpur', with 1.78–1.82 kg SS·box^{-1} [37,38], but inferior to those of the fruit of 'Shamouti Masry' trees grafted on 'Swingle', 2.98 kg SS·box^{-1} [75].

Together, the trifoliate orange-related rootstocks tested in this work, 'Swingle' and 'C-13', enhanced the 'Shamouti' cultivation under the humid subtropical climate by regulating tree size and fruit yield and maintaining fruit and juice quality. Both rootstocks may potentially increase the genetic diversification and horticultural performance of the tested scion in new orchards under similar soil–climate conditions.

5. Conclusions

In our study, we found that the horticultural traits of 'Shamouti' sweet orange trees are affected by the rootstock. Trees grafted on 'Sunki' and 'Cleopatra' mandarins are the most vigorous. In contrast, the ones on 'Swingle' citrumelo and 'C-13' citrange have moderate vegetative growth, which favors the use of these rootstocks in high-density plantings. 'Shamouti' trees are graft-compatible with all tested rootstocks, as no tree decline was observed 12 years after planting.

Despite having the shortest tree and smallest canopy size, 'Swingle' and 'C-13' genotypes provide higher yields to the 'Shamouti' trees, resulting in excellent yield efficiency. On the other hand, trees on 'Cleopatra' bear low fruit load and exhibit poor yield efficiency. Fruit produced by 'Shamouti' trees grafted on all tested rootstocks show excelled quality, meeting the commercial grading for the fresh fruit market.

Overall, among the tested rootstocks, 'Swingle' and 'C-13' are the most suitable rootstocks for 'Shamouti' sweet orange trees under the Brazilian humid subtropics and similar regions, as these rootstocks induce higher production to the scion and appropriated tree size, allowing higher tree density in new plantings. Further research and technological improvements will hopefully allow the intensification of cultivation of these citrus scion–rootstock combinations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111782/s1, Figure S1: Weather conditions (rainfall, average, maximum and minimum air temperature) from the experimental area in Londrina, state of Paraná, Brazil, for the 2009–2015 period (source: IDR-Paraná, 2022); Table S1: Soil chemical analysis from the experimental area in Londrina, state of Paraná, Brazil.

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