Long-term agronomical performance and iron chlorosis susceptibility of several *Prunus* rootstocks grown under loamy and calcareous soil conditions

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Abstract

The objective of this work was to evaluate the agronomic performance (vigor, yield, yield efficiency, number of root suckers), fruit quality (fruit weight, fruit size, flesh firmness, soluble solids content, and titratable acidity), leaf and fruit mineral nutrition (macro and micro elements), leaf chlorophyll concentration and iron chlorosis susceptibility of 'Big Top' nectarine cultivar grafted on 20 *Prunus* rootstocks and grown in loamy and calcareous soil under the hot climate conditions of the Ebro river basin (Spain). After the 10 years of the study (at 11th leaf), statistical analysis showed significant differences among rootstocks for most of the traits evaluated. Based on vigor and cumulative yield, 'Big Top' trees from Padac-04.03 rootstock were found to be the most vigorous and productive, followed by Castore, GF-677, Ishtara®, PS and Rootpac® 70. However, the most efficient rootstocks were Controller 5, Adesoto® 101, Rootpac® 40, Krymsk® 1, Ishtara®, Penta, IRTA-1, Polluce, and Padac-150. 'Big Top' fruits from Rootpac® 40 had the highest fruit weight and fruit size (>70 mm), with good soluble solids content and titratable acidity, but less firmness than the other 'Big Top' fruits. After 3 months with no application of chelate, chlorosis symptoms were visible in most of the trees, with those from Krymsk® 1 and PS showing the highest susceptibility. In contrast, AD-105, Adesoto® 101, Cadaman®, GF-677, Padac-150, Rootpac® 40 and Tetra were the least susceptible rootstocks. Controller 5, IRTA-1, Padac-04.03 and Penta had moderate susceptibility. In conclusion, Rootpac® 40, Ishtara®, IRTA-1 and Padac-150 may represent a good compromise between canopy size control, yield, yield efficiency, fruit size, and susceptibility to iron chlorosis.

Keywords: yield production, fruit quality, chlorophyll concentration, mineral elements, iron chlorosis

Introduction

The correct identification of rootstock × cultivar combination is a key requirement in activities associated with orchard production and management. In the Mediterranean area, almond × peach hybrid rootstocks are widely used (Zarrouk et al., 2006; Iglesias et al., 2018). Because many peach orchards located at the Ebro river basin area grown on calcareous and alkaline soils, which favor the occurrence of Fe chlorosis (Fernández et al., 2011), GF-677 is the most commonly used almond × peach hybrid rootstock in Spain (50% of the total rootstocks used in peach orchards) and across the Mediterranean area. GF-677 rootstock is tolerant to calcareous soil and lime-induced Fe chlorosis and has a good performance - particularly in soils with poor fertility, low water availability and high $CaCO_3$ content - and good graft compatibility with peach cultivars (Giorgi et al., 2005; Moreno et al., 1994; Nadal et al., 2013; Iglesias et al., 2018). Nevertheless, GF-677 is not recommended for very fertile soils or high planting densities, susceptible to root asphyxia, and is extremely vigorous, especially on early peach cultivars (Reighard and Loreti, 2008). Garnem is the second most planted rootstock in Spain (21% of the total rootstocks), followed by plum rootstocks (12%), Montclar or GF-305 (9%), Cadaman® (7%) and Rootpac® R (2%) (Iglesias et al., 2018). Garnem, a cross between almond and peach, with similar vigor to GF-677, is selected for its tolerance to iron chlorosis (similar to GF-677) and drought conditions, as well as its root-knot nematode resistance and good compatibility with almond and peach (Felipe, 2009). Among the plum rootstocks, special mention should be made of the plum rootstock Adesoto® 101, which is selected for its lower vigor compared to GF-677 (around 30-40% of vigor reduction), good adaptation to heavy and calcareous soil conditions, tolerance to iron chlorosis and root asphyxia, and resistance to several species of root-knot nematodes (Moreno, 2004; Font i Forcada et al., 2014). Montclar and GF-305 are both *Prunus persica* seedling rootstocks which induce high vigor in peach cultivars, are sensitive to iron chlorosis and show good compatibility with peach and nectarine cultivars. Montclar shows a better uptake of magnesium from the soil, but both are very susceptible to waterlogging (as is GF-677), *Agrobacterium*, *Phytophthora*, nematodes and some viruses (Reighard and Loreti, 2008). Cadaman®, with lower vigor compared to Garnem, is selected for its root-knot nematode resistance, good compatibility with almond and peach cultivars, and its higher yield efficiency and fruit size compared to

GF-677 (Iglesias and Carbó, 2006; Iglesias et al., 2018; Font i Forcada et al., 2012). Finally, Rootpac® R is selected for its resistance to root-knot nematodes and high tolerance to root asphyxia (Pinochet, 2010).

Control of tree vigor is becoming increasingly important for peach production. Unlike apple and pear, there are no widely acceptable size-controlling rootstocks for peach (Caruso et al., 2014) which have been adapted to limiting conditions such as root asphyxia, salinity, replant disease or active limestone in the soil. Worldwide, *Prunus* rootstock breeders are continuously searching for new rootstocks, preferably with medium-low vigor to allow the transition from traditional open-vase systems (5 m x 2.5-3.0 m, around 770 trees ha^{-1}) to high-density systems with smaller closely spaced trees (1,200–3,000 trees ha^{-1}) and bidimensional canopies which are more efficient in terms of yield, fruit quality, labor accessibility, mechanization and pest and disease treatments (Iglesias 2019). In addition, new rootstocks should be adaptable to a wide range of soil types and climatic conditions, and offer better tolerance/resistance to viruses, soil pests, diseases and iron chlorosis (Zarrouk et al., 2005; Jiménez et al., 2008; Gonzalo et al., 2012; Mestre et al., 2017). Among those conferring lower vigor than GF-677 and Cadaman® to peach cultivars, of particular importance are the commercial rootstocks including the Controller series from the University of California (Reighard et al., 2015), the dwarfing almond × peach rootstocks Castore and Polluce from the University of Pisa (Loreti and Massai, 2006a), the plum rootstocks Tetra and Penta from CREA Rome (Nicotra and Moser, 1997), and the Rootpac® 20 and Rootpac® 40 from Spain (Iglesias et al., 2018). However, very little or no information has been published on these rootstocks when grown under our pedo-climatic conditions. Therefore, the primary purpose of this work is to compare the influence of 20 *Prunus* rootstocks - some already released and others under selection, with control vigor, genetic background, and origin - on productive parameters, leaf and fruit mineral nutrition, fruit quality and iron chlorosis susceptibility on ‘Big Top’ nectarine cultivar grown over 12 years under loamy and calcareous soil conditions typical of the Ebro river basin area.

Material and Methods

Plant material, site description and experimental design

The study was carried out over eleven growing seasons (2008-2018) at an experimental orchard of the IRTA Fruitcentre (Gimenells; NE Spain; 41º 39’ 18.77” N and 0º 23’ 31.41”

E). The mid-season nectarine ‘Big Top’, a yellow flesh cultivar released by Zaiger Genetics Inc., was selected for use as it is the most planted and popular nectarine in Europe and the reference cultivar (Reig et al., 2012, 2015, 2016). The attributes of the ‘Big Top’ nectarine cultivar include its intense and early red color, sweet taste, slow softening and excellent postharvest storage potential (Iglesias and Echeverría, 2009; Reig et al., 2017). Twenty rootstocks from different genetic origins were evaluated (Table 1). Cadaman® and GF-677 rootstocks were introduced in the trial as rootstock references.

Dormant bud trees were planted in winter 2008 on Aquic Xerofluent soil (Table 2). Rootpac® 40 was planted in winter 2009, and Controller 5 and Controller 9 in winter 2010. Trees were trained with the Catalan vase system, a relatively small and easy-to-train form, spaced at 5 m x 2.6 m (Montserrat and Iglesias, 2011). Fertilizers were applied by drip irrigation, and foliar micronutrients, pesticides and insecticides were applied as necessary, following industry standards. Trees grew under a cold semiarid Mediterranean climate (Bsk in the Köppen-Geiger climate classification system). The area has around 300-500 mm annual rainfall, and 32 ºC mean summer daily temperature.

The experiment was established in a randomized block design with four blocks, with the base plot consisting of three trees per scion-rootstock combination. The central tree of each base plot was used for the study.

2008-2018 Seasons

In order to compare all rootstocks at the same age or leaf, for Rootpac® 40 the horticultural and fruit quality assessment data, described below, relative to the 11th leaf were estimated, and for Controller 5 and Controller 9 the 10th and 11th leaf data were estimated as well.

Field assessments

From the third year after planting (3rd leaf) onward (to 11th leaf), we recorded the following parameters for each scion × rootstock combination. Trees were harvested in two different picks separated by 4-7 days. The criteria stablished for the first pick were: fruit size ≥60 mm and fruit color ≥80% of fruit surface, corresponding to a flesh firmness in the range of 40-50 N (Table 3). After each of the two picks per season, the whole yield of each controlled tree was graded for fruit size and weight using a commercial electronic fruit grader (MAF RODA Iberica, Alzira, Spain). Total yield per tree, average fruit weight and total number of fruits per fruit size (<60 mm, 60-65 mm, 65-70 mm, 70-75 mm, and >75 mm) were then calculated for each pick. At the end of each season, tree circumference was recorded at 20 cm above the graft union, and the trunk cross-sectional area (TCSA, cm^2) was then calculated. Cumulative yield (CY), cumulative yield efficiency (CYE, kg/cm^2 and number of $fruits/cm^2$), fruit weight (g) and fruit size (mm) of each scion × rootstock combination were computed from 2010 to 2018. Root suckers were removed each year, and during the last three years of the study (2016, 2017, and 2018) they were counted and removed thereafter.

Fruit quality assessments

From the third year after planting onward and after calibration, a sample of 30 fruits for each scion × rootstock combination and harvest was used for fruit quality determinations. Flesh firmness (FF), soluble solids content (SSC) and titratable acidity (TA) were measured with a Pimprenelle robotic laboratory (Setop, Cavaillon, France). FF was expressed in N, SSC in ºBrix, and TA in g malic acid L^{-1}.

Leaf and fruit mineral elements assessment

Leaf and fruit mineral concentrations were determined in 2015 and 2016 for ‘Big Top’ trees. Leaf sampling was carried out at 120 days after full bloom (DAFB). Leaf samples (30 fully expanded and mature leaves per tree) were collected from the central part of each shoot and around the crown of the trees. The leaves were sent to an external laboratory for nutrient content quantification. All elements were obtained by inductively coupled plasma mass spectrometry (ICP-OES), except for N which was determined by Kjeldahl analysis (Gerhardt-Vapodest, Germany). Concentrations were expressed as mg 100 g^{-1} (N, P, K, Ca, and Mg) and as mg kg^{-1} (B, Fe, Zn, Cu, and Mn), all on a dry weight basis.

Leaf chlorophyll assessment

The chlorophyll (Chl) concentration per unit leaf was determined in 2016 and 2017 for ‘Big Top’ trees under standard fertirrigation conditions in the field using a SPAD-502 meter (Minolta Co., Osaka, Japan), as described in other *Prunus* rootstock studies (Mestre et al., 2015, 2017). Peryea and Kammereck (1997) proposed that the green color of the leaf, assessed with a SPAD (soil and plant analyzer development) chlorophyll meter, served as an unbiased quantitative measure of the severity of leaf chlorosis associated with Fe deficiency and of the relative effectiveness of Fe fertilization treatments. Measurements on 30 leaves of bearing shoots (at the middle section of the leaf, midway between the central vein and the leaf edge) per tree at the same height and development stage were carried out at 120 DAFB.

2019 Season

Leaf chlorophyll and iron chlorosis assessments

From mid-April to the end of June, 30 random leaves were selected on a biweekly basis at the same height and development stage, and SPAD measured.

After the fruit set phenological stage, no iron chelate was applied in order to induce iron chlorosis. The chlorosis incidence of each rootstock was characterized visually (a subjective method, but simple, economic, and fast) on a biweekly basis from mid-April to the beginning of July, according to a chlorosis scale (Sanz and Montañés, 1997): 0, no symptoms; 1, incipient symptoms as in very light interveinal chlorosis in some apical leaves; 2, incipient chlorosis symptoms in young leaves (interveinal yellowing); 3, interveinal chlorosis symptoms in both young and mature leaves; 4, tree with yellowish white young leaves and some necrotic areas, and the rest of the leaves yellowish green; and 5, tree with defoliated and dead growth buds, and all leaves yellowish with necrotic areas.

Field and fruit quality assessments

Harvest date was determined on the basis of FF, ranging from 40-50 N, fruit size and fruit color. Trees were harvested in two different picks separated by 7 days. Fruits were graded for fruit size and weight as described above. At the end of the season, tree circumference was recorded at 20 cm above the graft union, and the TCSA (cm^2) was then calculated.

The FF, SSC and TA were evaluated as described above.

Statistical analysis

An ANOVA was performed using JMP (Version 12; SAS Institute Inc., Cary, NC, USA). Means were separated by Tukey's HSD test ($P \leq 0.05$). Pearson's correlation coefficients were applied to examine relationships between parameters.

Results

2008-2018 Seasons

Field assessments

At the eleventh year after planting, tree vigor (expressed as TCSA) showed important differences attributable to rootstock (Figure 1, Table 3). Based on vigor and cumulative yield, 'Big Top' trees on Padac-04.03 were the most vigorous and productive, but no significant differences were observed when compared with Castore, GF-677, Ishtara®, PS and Rootpac® 70. In contrast, 'Big Top' trees on Controller 5, Controller 9, Krymsk® 1, and Polluce were the least vigorous and productive.

The corresponding cumulative yield production (2010-2018) percentage of the 1st and 2nd harvest picks is shown in Figure 2. In general, more than 50% of the fruits were harvested in the first pick, except for the reference rootstocks, Cadaman® and GF-677. Rootpac® 40 had the highest 1st pick incidence, followed by Pacer-01.36, Rootpac® 20 and Tetra. The plum rootstocks AD-105, Adesoto® 101, Padac-150, and Penta, and the interspecific hybrid Ishtara® also had high 1st pick percentage values.

All rootstocks produced fruits from the third leaf onwards (Figure 3), showing clear significant differences between rootstocks at the 4th leaf. The most vigorous rootstocks, Padac-04.03, Castore, GF-677 and Rootpac® 70 had higher yield compared to the other rootstocks across the years, while the least vigorous had the lowest values. In this case, because spacing was the same for all rootstocks, higher yields per tree or per hectare are related with rootstock vigor and greater canopy volume. The ideal at "posteriori" to compare yields should be to recalculate the planting distance of each rootstock based on it vigor induced and considering GF-677 as the reference. Hence, comparing them in terms of yield efficiency (kg cm^{-2} and number of fruits per cm^2), Controller 5 had the highest value, although it did not significantly differ from Adesoto® 101, Rootpac® 40, Krymsk® 1, Ishtara®, Penta, IRTA-1, and Padac-150. The lowest yield efficiency was recorded for PS, followed by Rootpac® 70 (Table 3).

Sensitivity to root sucker emission (Table 3) was high for Pollizo, AD-105, Krymsk® 1 and Pacer-01.36, and low for Controller 5, Rootpac® 70 and Polluce.

Fruit quality assessments

Fruit quality parameters are shown in Table 4. 'Big Top' fruits from Padac-150, PS, Tetra, and GF-677 had the highest FF values, and those from Controller 9 and Rootpac® 40 the lowest. This could be related to the early ripening induced by these and other rootstocks, also seen in the high average yield harvested in the first pick (Figure 2). The highest SSC values were observed in fruits from Krymsk® 1 and IRTA-1, while Rootpac® 40 and Rootpac® 70 had the lowest values. In any case, all values are >10ºBrix, the minimum required for most export markets. As expected, those rootstocks that induced higher firmness also induced higher acidity, as for example in Tetra.

Average fruit weight was significantly affected by rootstock (Table 4). Rootpac® 70, followed by Ishtara® and Rootpac® 40, induced the biggest fruits, and Controller 5, Controller 9, Krymsk® 1 and PS the lowest.

Based on the cumulative fruit size distribution by intervals, the predominant fruit size was 65-70 mm, followed by 70-75 mm (Figure 4). In fruit size 65-70 mm, no significant differences between rootstocks were observed. The peach × almond rootstock Rootpac® 40 and the plum rootstock Padac-150, followed by Rootpac® 70 and Ishtara®, had the highest percentage of fruits in the 70-75 mm fruit size. However, considering the most interesting fruit size in terms of category (A and AA categories), and consequently the return price for growers (>70 mm), Rootpac® 40, followed by Rootpac® 70, Padac-150 and Penta had the highest percentage of fruits greater than 70 mm in size. In fact, the first three of these rootstocks, and in particular Rootpac® 40, had the highest average fruit size (Table 4).

Leaf and fruit mineral elements assessment

Mineral elements were significantly affected by rootstocks in both leaf and fruit tissues, except P and Fe for leaves, and N and Mg for fruits (Tables 5 and 6).

In terms of macro elements, Krymsk® 1 and Rootpac® 20 had significantly higher leaf N concentration than the other rootstocks, except with respect to AD-105, Cadaman®, Controller 5, Pacer-01.36, Padac-150, Polluce and PS (Table 5). The highest leaf K concentrations were obtained in the plum rootstock Tetra, the peach × plum rootstock PS, and the interspecific hybrid Rootpac® 70, followed by AD-105, Penta, Polluce, and Rootpac® 40 (Table 5). The other rootstocks were within the range of optimal values. The highest leaf Ca concentrations was found in Controller 9, and the lowest in Krymsk® 1 (Table 5). The highest leaf Mg concentrations were obtained in Cadaman® and Rootpac® 70, and the lowest in Krymsk® 1 (Table 5).

In terms of micro elements, the highest Mn leaf concentration was observed in Penta, although with no significant differences from the other rootstocks except for Cadaman®, IRTA-1, Polluce , PS, Rootpac® 40, and Rootpac® 70 (Table 6). PS and Rootpac® 70 had the highest leaf B concentration values, and Controller 5 and IRTA-1 the lowest (Table 6).

The PS rootstock, followed by Tetra and Krymsk® 1, had the highest fruit P concentration, and Ishtara® the lowest. The highest fruit K concentration values was for AD-105, although it did not differ significantly from the rest of the rootstocks except for Controller and Padac 04-03 (Table 5). PS, IRTA-1 and AD-105 rootstock had the highest fruit Ca value, and Cadaman® and Adesoto® 101 the lowest. The highest fruit Fe values were for Tetra, and the lowest for Rootpac® 20 (Table 6). Penta and Rootpac® 40 had the highest fruit Mn values, although they did not differ significantly from the other rootstocks except for Controller 5, Controller 9, Ishtara®, Krymsk® 1 and Rootpac® 70 (Table 6). Finally, Rootpac® 70 had the highest fruit B values, and Padac-150 the lowest (Table 6).

Physiological assessment

Leaf SPAD readings (2015 and 2016), on average, showed no significant differences between rootstocks, except for PS, Rootpac® 40 and Rootpac® 70 (Figure 5). Despite the differences, no iron deficiency was observed in the rootstocks in those two years.

2019 Season

Leaf chlorophyll and iron chlorosis assessments

In order to evaluate the sensitivity of different rootstocks to iron induced chlorosis, in 2019 at the end of the trial, no iron chelate was applied after fruit set (the end of March). Different levels of rootstock susceptibility were observed in both apical and expanded leaves (Figure 6), with SPAD values also decreasing over time (Table 7). In fact, a high negative correlation was observed between symptomatology and SPAD values ($r = -0.81$, $P \leq 0.05$). During the first month of evaluation, in general, most of the rootstocks presented no or very few chlorosis symptoms, except one tree from Controller 9, Krymsk® 1, Polluce and Rootpac® 40, and three of the four trees from PS rootstock. One month later, some trees from AD-105, Adesoto® 101, Castore, GF-677, Padac-150 and Tetra showed incipient symptoms of chlorosis. At the third month of evaluation, after the pit hardening stage and during fruit growth, chlorosis symptoms were more visible in most of the trees, with the trees from Krymsk® 1 and PS, and some trees from Ishtara®, Pacer-01.36, and Rootpac® 20 showing the highest degree of susceptibility. In contrast, AD-105, Adesoto® 101, Cadaman®, GF-677, Padac-150, Rootpac® 40 and Tetra were the least susceptible rootstocks after 3 months without application of iron chelate. Controller 5, IRTA-1, Padac-04.03 and Penta showed moderate susceptibility.

Field and fruit quality assessments

Cadaman® and GF-677 had the highest yield in 2019, although they did not differ statistically from the rest of the rootstocks, except for Krymsk® 1 (Table 8). Rootpac® 40 induced the largest fruits in weight, whereas PS had the lowest fruit size. Low and positive significant correlations were found between SPAD values and yield ($r = 0.35$, $P \leq 0.001$) and SPAD and fruit weight ($r = 0.40$, $P \leq 0.001$), whereas low and negative correlations were found between chlorosis and yield ($r = -0.31$, $P \leq 0.001$) and chlorosis and fruit weight ($r = -0.44$, $P \leq 0.001$).

Based on fruit size distribution (Figure 7), the predominant fruit size was, in general, 65-70 mm, followed by 60-65 mm. After three months, in general, those rootstocks with low incidence of chlorosis had the highest percentage of fruits in the fruit size distribution 65-70 mm, namely Castore, Rootpac® 70, IRTA-1, and Pacer-04.03. The rootstock which induced the largest average fruit size and consequently the fruit with the highest commercial value (>70 mm) was Rootpac® 40, followed by Adesoto® 101, Padac-04.03, Cadaman® and IRTA-1. However, the rootstocks most affected by iron chlorosis, PS and Krymsk® 1, also had the highest percentage of fruits in the <60 mm and 60-65 mm ranges, and the lowest percentage in the >70 mm range. In fact, chlorosis symptoms from the last evaluation were correlated positively with the size distribution <60 mm ($r = 0.39$, $P \leq 0.001$) and 60-65 mm ($r = 0.27$, $P \leq 0.05$), and negatively with the size distribution 65-70 mm ($r = -0.33$, $P \leq 0.05$) and 70-75 mm ($r = -0.26$, $P \leq 0.05$).

The fruit quality parameters that were considered in this study are shown in Table 8. 'Big Top' fruits from Cadaman®, Ishtara®, PS and GF-677 had the highest FF values, while the lowest were from Krymsk® 1. This last rootstock, however, had the highest SSC value. Analyzing all rootstocks together, the fruit quality parameters (FF, SSC and TA) showed no significant correlations with chlorosis or SPAD values from the last evaluation (27th June).

4. Discussion

In the Ebro Valley region where the trial was carried out, using the same training system and applying the same cultural practices (fertirrigation, etc.) to all the rootstocks considered in the study and evaluated in a warm climate and under loamy and calcareous soil conditions, significant differences were found between *Prunus* rootstocks in field traits, leaf and fruit mineral elements, fruit quality and susceptibility to iron chlorosis.

Padac-04.03 and Rootpac® 70 were the most vigorous and productive rootstocks in terms of cumulative yield, but with low yield efficiency in agreement with previous *Prunus* rootstock studies (Zarrouk et al., 2005; Jiménez et al., 2011; Ben Yahmed et al., 2016). The invigorating rootstock Rootpac® 70 and the medium-low vigor rootstock Rootpac® 40 produced high average fruit weight and fruit size values in agreement with other authors (Jiménez et al., 2011; Ben Yahmed et al., 2016; Iglesias et al., 2018). These results do not support the hypothesis of a competition between vegetative growth and fruit growth, principally for the available photosynthate. For the mid-season cultivar 'Big Top', a tree vigor increase, via grafting on a vigorous rootstock, probably enhances the translocation of photosynthate to the maturing fruit and thus stimulates its enlargement (Bussi et al., 1995).

In relation to medium rootstock vigor, some authors (Jiménez et al., 2011; Reig et al., 2016) have reported similar vigor for Tetra and GF-677, which concurs with our results. Caruso et al. (2014) reported growth reductions of the early-ripening 'Tropic Snow' peach tree grafted on Castore at 6th leaf when compared to GF-677. While our results at 6th leaf showed the same trend, at 11th leaf Castore had similar vigor to GF-677 when grafted on the mid-season nectarine 'Big Top'.

For decades, a more efficient production system has been considered a priority for the peach industry in Spain. The Catalan vase training system is nowadays the most commonly used system because of its low cost in terms of orchard establishment (low planting density, no support structure, partial mechanization) and the availability of paclobutrazol for vigor control (Montserrat and Iglesias; Iglesias et al., 2018). Medium-low vigor rootstocks for use with peach do exist commercially, but their use is very limited in warm Mediterranean environments (Loreti and Massai, 2006b). Their main drawbacks are the excessive need for chill units, a lack of compatibility with many peach and nectarine cultivars (in the case of plums and plum hybrids), and susceptibility to iron chlorosis and soil-borne pathogens, such as fungi and root-knot nematodes, so common in many peach-growing regions of Spain (Pinochet, 1997; Iglesias et al., 2018). Controller 5 was the least vigorous rootstock, inducing high yield efficiency when compared with the other rootstocks, in agreement with other studies (Reighard et al., 2011). Nevertheless, its lower cumulative yield, fruit weight and fruit size, as reported by Reighard et al. (2011) in several U.S. locations, do not make it suitable for peach orchards with open vase as a training system. In contrast, Rootpac® 40, with its induced medium vigour, high yield efficiency and good accumulated yield as well as good fruit size and fruit weight, may be a good option for establishing more efficient and sustainable peach production systems in regions where high density orchards are not feasible due mainly to the lack of adequate genetic material (Jiménez et al., 2011; Iglesias et al., 2018; Iglesias, 2019). In addition, the advance of ripening induced by Rootpac® 40 it's a key for profitability in early producing areas and precocious harvest varieties. Low vigor and high yield efficiency, together with high fruit quality, are the ideal parameters for high density peach orchards, as has been the case for apple and pear all around the world in recent decades. This raises the possibility of establishing pedestrian and/or bidimensional orchards, with more accessible canopies for the workers and better adaptation to mechanization, resulting in reduced labor costs, especially at thinning, pruning and harvest (Jiménez et al., 2011; Iglesias, 2019).

The decreasing yield trend of Krymsk® 1 from 6th leaf onwards is a result of its graft incompatibility with the 'Big Top' nectarine cultivar. Zarrouk et al. (2006) reported 'translocated' or 'localized' graft incompatibility when Krymsk® 1 was grafted with 29 peach cultivars. Reighard et al. (2011) reported scion incompatibility of Krymsk® 1 grafted on different peach cultivars at few U.S. locations. However, Jiménez et al. (2011) did not report any graft incompatibility when 'Calanda' peach was grafted on Krymsk® 1 and field performance evaluated over 7 years. The lack of affinity of Krymsk® 1 affects plant growth and development, decreases orchard productivity over time and causes the death of adult plants (Barreto et al., 2017). Besides scion incompatibility, some *Prunus* rootstock studies (Reighard et al. 2011, 2015) also reported high root suckering on Krymsk® 1 in California and other U.S. States, in agreement with our results. Root suckering is an important trait for growers because of its impact on orchard management.

Several studies have reported the different influence of *Prunus* rootstocks on the nutrient content in leaves (Reighard et al., 2013; Mestre et al., 2015, 2017; Jimenes et al., 2018), but the scion and the environmental conditions also affect nutrient absorption and translocation (Ballesta et al., 2010). However, kinetic parameters related to nutrient uptake efficiency are not typically considered for *Prunus* rootstock selection, such as nitrogen (N) forms nitrate (NO3-) and ammonium (NH4+), as N is the nutrient that most affects growth, yield and fruit composition (Zhang et al., 2016). For 'Big Top' trees cultivated in loamy and calcareous soil, nitrogen was affected more by genotype than by tree vigor. Nitrogen uptake was not limiting for any rootstock, with all rootstocks showing optimal N values according to the reference values (Villar and Arán, 2008) except for Krymsk® 1 and Rootpac® 20, which had slightly higher than optimal values. Leaf K content ranging from 15-25 g kg^{-1} is considered adequate for peach trees cultivated in Spain (Villar and Arán, 2008). In this study, some rootstocks had slightly higher than optimal values, as was the case for AD-105, IRTA-1, Penta, Polluce, PS, Rootpac® 40, Rootpac® 70, and Tetra. The high Tetra leaf K value concurs with the results of Mestre et al. (2015), who reported higher than optimal leaf K values of 'Big Top' trees grafted on Tetra and grown under heavy and calcareous soil conditions. Most of the rootstocks evaluated in the present study had leaf Ca values within the optimal range, except for Controller 9. This rootstock was more efficient in absorbing and translocating this nutrient in leaves than the rest of the rootstocks. The Mg, Mn, Zn, B, and Cu leaf contents detected in all rootstocks were in accordance with the range considered optimal (Villar and Arán, 2008), except for Rootpac® 70 which had lower than optimal leaf Mn values.

As the thresholds for fruit mineral element concentrations in mature tissue have been defined for macro elements, but not microelements (Villar and Arán, 2008), only the optimal macro element values are considered in this study. The N and P fruit contents were slightly higher than the optimal values (Villar and Arán, 2008) for most of the rootstocks, except for Ishtara® and Pacer-01.36 in N fruit content, and for Cadaman®, Controller 9, IRTA-1, Ishtara®, Pacer-01.36, Padac-04.03, Rootpac® 40 and Rootpac® 70 in P fruit content. K is the most abundant element in the fruits, providing an appropriate size, balanced flavor and more intense coloration (Jimenes et al., 2018). The higher than optimal K levels (Villar and Arán, 2008) detected in 'Big Top' fruits from all rootstocks might be explained by competition between K and Ca (Reighard et al., 2013; Jimenes et al., 2018) impairing absorption of Ca, which had a lower than optimal content (Villar and Arán, 2008). Since Ca is very important for peach fruit quality, rootstocks that negatively impact fruit Ca levels require postharvest storage and testing to determine if fruit firmness is reduced (Reighard et al., 2013), which was not ascertained in this study. Finally, all rootstocks showed a higher than optimal fruit Mg content (Villar and Arán, 2008).

Regarding fruit quality, our results are in agreement with other 'Big Top' rootstock trials (Reig et al., 2016). All rootstocks exhibited acceptable fruit quality. 'Big Top' fruits from all rootstocks achieved 12º Brix over the several years of the study (2008-2018), except for 2019 when Cadaman®, Rootpac® 40 and Rootpac® 70 induced 'Big Top' fruits with values which, although below 12º Brix, were nevertheless commercially acceptable.

Iron chlorosis is chiefly associated with plant growth in high pH, calcareous soils, and with the presence of high bicarbonate concentrations which can inhibit Fe uptake mechanisms (Eichert et al., 2010; Nadal et al., 2013). Different approaches can be used to control Fe chlorosis in tree crops. The genetic approach to prevent iron chlorosis is based on the use of tolerant rootstocks (Iglesias and Carbó 2006; Jiménez et al., 2011; Gonzalo et al., 2012), whereas the agronomic approach is to apply iron chelate treatments, which substantially increases orchard management costs (Iglesias et al., 2018).

In our trial, over 10 years we used both approaches and with the same iron chelate dose applied each year for all *Prunus* rootstocks (around 15 $kg.ha^{-1}$ of ortho-ortho EDDHA 5.25% Fe), with no significant symptoms of iron chlorosis observed among rootstocks. The indirect measurement of leaf chlorophyll concentration by SPAD readings used as an indicator of iron chlorosis tolerance in *Prunus* trees during two consecutive seasons (2015 and 2016) confirmed these observations, with SPAD mean values over 35 and non-statistical differences between rootstocks. In addition, Adesoto® 101, Rootpac® 40, Rootpac® 70, and Tetra presented similar SPAD values to previous plum and peach trials established on calcareous soils (Zarrouk et al., 2006; Jiménez et al., 2011).

In 2019, with no iron chelate application, a phenotypic analysis, using two different parameters to determine the occurrence and severity of iron chlorosis, provided a precise dataset to determine the most tolerant rootstock to iron deficiency in the soil conditions of the trial. The high correlation between the two parameters supports their use as an indicator of *Prunus* rootstock susceptibility to iron chlorosis over time. Chlorosis occurrence in peach has been associated with deceased yield and quality, and delayed fruit ripening (Gonzalo et al., 2012). This harmful nutritional disorder is a problem of economic significance because crop quality and yields can be severely affected. In this *Prunus* rootstock study, yield and fruit size were negatively correlated with iron chlorosis symptoms. However, in general, no correlation was found with 'Big Top' fruit quality, indicating that fruit quality may be more affected by rootstock than iron chlorosis symptoms.

5. Conclusion

Significant differences among the 20 rootstocks were found in most of the traits evaluated. In the assessment of agronomic traits, significant differences were observed in tree vigor and yield efficiency, sensitivity to sucker emission, fruit quality, and susceptibility to iron chlorosis. In view of the possibility of further EU growth bioregulator limitations and the need for greater input use efficiency in terms of labor, treatments and mechanization through orchard intensification, Rootpac® 40, Ishtara®, IRTA-1 and Padac-150 may represent a good compromise between canopy size control, yield, yield efficiency, fruit size and susceptibility to iron chlorosis. Consequently, one or more of these rootstocks could be an interesting alternative to GF-677 for the cultivation of peach in warm climates and calcareous soils.

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Table 1. List of studied rootstocks.

Rootstock	Species	Breeder[a]
AD-105	*P. insititia (open pollination of Adesoto)*	CSIC, Spain
Adesoto® 101	*P. insititia (open pollination of Adesoto)*	CSIC, Spain
Cadaman® Avimag	*P. persica x P. davidiana*	INRA, France-Hungary
Castore	*P. amydalus x P. persica*	Pisa University, Italy
Controller 5	*P salicina x P. persica*	California University, USA
Controller 9	*P salicina x P. persica*	California University, USA
INRA® GF-677	*P. amygdalus x P. persica*	INRA, France
IRTA-1	*P. amygdalus x P. persica*	IRTA, Spain
Ishtara® (Ferciana)	*(P. cerasifera x P. salicina) x (P. cerasifera x P. persica)*	INRA, France
Krymsk® 1 (VVA1)	*P. tomentosa x P. cerasifera*	E.E. Krasnovar
Pacer-01.36	*(P. cerasifera x P. spinosa) x(P. spinosa x P. persica)*	AI, Spain
Padac-150	*P. insititia*	CSIC-AI, Spain
Padac-04.03	*P. cerasifera x (P. amygdalo x P. persica)*	CSIC-AI, Spain
Penta	*P. domestica*	CREA Rome, Italy
Polluce	*P. amydalus x P. persica*	Pisa University, Italy
PS	*P. persica x P. cerasifera*	Battistini Vivai, Italy
Rootpac® 20	*P. besseyi x P. cerasifera*	AI, Spain
Rootpac® 40 (Nanopac)	*(P. amydalus x P. persica) x (P. amydalus x P. persica)*	AI, Spain
Rootpac® 70 (Redpac)	*(P. persica x P. davidiana) x (P. amygdalus x P. persica)*	AI, Spain
Tetra	*P. domestica*	CREA Rome, Italy

[a] AI = Agromillora Iberia S.L. nursery company, Spain; CREA = Consiglio per la ricerca in agricoltura l'analisi dell'economia agrarian; CSIC = Consejo Superior de Investigaciones Científicas; INRA = Institut National de la Recherche Agronomique; IRTA = Institut de Recerca i Tecnologia Agroalimentàries

Table 2. Soil analysis description.

Depth (cm)	Texture	E.C. (1:5) (dS/m)	pH	Organic Matter (%)	P (ppm)	K^c (ppm)	N-NO_3 (ppm)	Mg (ppm)	$CaCO_3$ (%)	Ca (ppm)
0-35	loam	0.52	8.00	3.01	2.00	425	128	258	19	7461
35-60	loam	0.31	8.10	2.44	21.0	160	79	175	18	7525
60-90	loam	0.40	8.20	1.01	4.0	62	22	177	31	7802

Table 3. Vigor, cumulative yield, yield, yield efficiency, and average root suckers at the eleventh year after planting (2018) of ‘Big Top’ nectarine grafted on 20 different *Prunus* rootstocks. Grey bars represent variable value.

Rootstock	TCSA (cm^{-2})	Cumulative yield (kg tree^{-1})	Yield (Kg tree^{-1})	Yield efficiency (kg cm^{-2})	Yield efficiency (nº fruits cm^{-2})	Average root sucker
AD-105	163.1 bcde	277.5 abcd	30.8 abcd	1.7 bcd	11.4 abc	11.2 a
Adesoto® 101	135.3 def	308.6 abc	34.3 bcde	2.3 ab	14.0 ab	4.6 bcde
Cadaman® Avimag	164.9 bcde	258.5 abcd	32.3 abcd	1.6 bcd	10.7 bc	4.4 cde
Castore	189.5 abcd	323.5 abc	35.9 abc	1.7 bcd	11.2 abc	4.8 bcde
Controller 5	86.4 f	158.9 d	18.2 f	2.8 a	17.6 a	1.3 e
Controller 9	173.2 def	190.7 cd	21.2 ef	1.4 bcd	8.6 bc	2.0 cde
INRA® GF-677	181.8 abcd	292.5 abc	33.4 abcd	1.6 bcd	10.5 bc	3.7 cde
IRTA-1	166.1 bcde	295.7 abc	32.8 abcd	1.8 abcd	11.7 abc	2.7 cde
Ishtara®	175.8 abcd	343.7 ab	38.2 ab	2.0 abcd	11.9 abc	4.8 bcde
Krymsk® 1	92.8 ef	198.3 cd	22.0 ef	2.1 abc	14.1 ab	10.6 ab
Pacer-01.36	158.2 cde	265.5 abcd	29.5 bcde	1.7 bcd	10.4 bc	7.9 abc
Padac-04.03	250.6 a	365.3 a	40.6 a	1.5 bcd	9.5 bc	4.1 cde
Padac-150	154.4 cde	272.3 abcd	30.2 bcde	1.8 abcd	10.7 bc	7.3 abcd
Penta	157.6 cde	306.2 abc	34.0 abcd	1.9 abcd	12.12 abc	3.3 cde
Polluce	121.5 def	217.6 bcd	26.4 cdef	1.8 bcd	11.1 bc	1.7 de
PS	226.3 abc	228.0 bcd	26.1 def	1.0 d	6.5 c	2.3 cde
Rootpac® 20	163.5 bcde	256.7 abcd	28.5 bcde	1.6 bcd	10.1 bc	2.5 cde
Rootpac® 40	164.6 bcde	294.4 abc	32.7 abcd	1.8 abc	12.1 abc	2.9 cde
Rootpac® 70	236.7 ab	305.7 abc	35.9 abc	1.2 cd	7.2 c	1.2 de
Tetra	169.8 bcde	259.1 abcd	28.8 bcde	1.5 bcd	9.5 bc	5.4 abcde
$P \leq 0.05$	***	***	***	***	***	***

Data were evaluated by one-way analysis of variance (ANOVA); *** $P \leq 0.001$; ** $P \leq 0.01$; *$P \leq 0.05$; ns, not significant. Means within a column followed by different letters denotes significant differences among treatments (Tukey's honestly significant difference, $P \leq 0.05$).

Table 4. Fruit quality parameters (from 6th leaf to 11th leaf), and fruit weight and fruit size (from 3rd leaf to 11th leaf) of ‘Big Top’ nectarine grafted on 20 *Prunus* rootstocks. Grey bars represent variable value.

Rootstock	FF (N)	SSC (ºBrix)	TA (g L^{-1})	Fruit weight[a] (g)	Fruit size[a] (mm)
AD-105	43.0 abcd	12.9 bcdef	5.3 ab	155.4 bcd	66.8 bcd
Adesoto® 101	40.9 cd	13.4 abcde	4.8 bc	167.2 abc	68.2 abcd
Cadaman® Avimag	44.9 abc	12.4 ef	5.1 ab	158.8 abcd	67.8 abcd
Castore	43.0 abcd	12.7 def	5.2 ab	157.5 abcd	67.5 abcd
Controller 5	44.8 abc	14.2 ab	4.8 bc	144.5 d	65.9 d
Controller 9	38.5 d	13.4 abcde	4.0 c	149.9 cd	67.3 cd
INRA® GF-677	45.5 ab	12.9 bcdef	5.2 ab	156.7 abcd	66.9 cd
IRTA-1	43.3 abcd	14.3 a	5.2 ab	155.2 bcd	67.1 bcd
Ishtara®	44.3 abc	12.8 bcdef	5.2 ab	173.5 a	68.1 abcd
Krymsk® 1	40.9 cd	14.6 a	4.9 abc	150.8 cd	65.3 d
Pacer-01.36	41.6 bcd	13.9 abcd	4.9 abc	161.8 abcd	67.5 abcd
Padac-04.03	43.1 abcd	12.4 ef	5.1 ab	164.7 abcd	68.3 abcd
Padac-150	46.7 a	13.9 abcd	5.3 ab	164.3 abcd	68.7 abc
Penta	42.9 abcd	12.7 def	5.1 ab	161.8 abcd	68.0 abcd
Polluce	43.2 abcd	13.3 abcde	5.1 ab	158.6 abcd	67.9 bcd
PS	45.7 ab	14.1 abc	5.3 ab	152.1 cd	66.3 cd
Rootpac® 20	41.6 bcd	13.7 abcde	4.9 abc	157.9 abcd	67.0 bcd
Rootpac® 40	39.7 d	11.9 f	4.9 abc	168.7 ab	70.5 a
Rootpac® 70	45.0 abc	11.9 f	5.6 ab	173.9 a	69.6 ab
Tetra	45.5 ab	13.4 abcde	5.7 a	162.8 abcd	68.3 abcd
$P \leq 0.05$	***	***	***	***	***

Data were evaluated by one-way analysis of variance (ANOVA); *** $P \leq 0.001$; ** $P \leq 0.01$; *$P \leq 0.05$; ns, not significant. Means within a column followed by different letters denotes significant differences among treatments (Tukey's honestly significant difference, $P \leq 0.05$).

[a] Weighted fruit size according to fruit size distribution (<60 mm, 60-65 mm, 65-70 mm, 70-75 mm, and >75 mm).

Table 5. Leaf and fruit macro elements of ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks at 120 days after full bloom. Mean values of 2015 and 2016 expressed as mg 100 g^{-1}.

Rootstock	N		P		K		Ca		Mg	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
AD-105	3.46 ab	131.9 a	0.21 a	27.4 abc	3.29 abcd	262.6 a	2.53 b	6.63 a	0.36 de	10.21 a
Adesoto® 101	3.28 bc	117.7 a	0.21 a	25.5 abc	2.97 abcdef	240.0 ab	2.54 b	5.00 b	0.37 cde	9.50 a
Cadaman® Avimag	3.47 ab	123.0 a	0.23 a	22.6 abc	2.72 cdefg	215.0 ab	2.72 ab	5.00 b	0.54 ab	8.64 a
Castore	3.31 bc	124.1 a	0.22 a	26.5 abc	3.02 abcd	215.8 ab	2.72 ab	6.51 ab	0.47 abc	9.10 a
Controller 5	3.39 abc	122.4 a	0.19 a	24.4 abc	2.29 g	227.3 ab	2.39 bc	6.43 ab	0.40 cd	9.59 a
Controller 9	3.2 bc	112.0 a	0.21 a	22.5 bc	2.40 fg	208.1 b	3.30 a	5.50 ab	0.47 abc	8.51 a
INRA® GF-677	3.29 bc	131.5 a	0.21 a	25.9 abc	2.64 efg	219.1 ab	2.85 ab	5.34 ab	0.44 cd	9.17 a
IRTA-1	3.31 bc	128.6 a	0.22 a	23.7 abc	3.32 abc	235.3 ab	2.47 bc	6.66 a	0.39 cde	9.77 a
Ishtara®	3.32 bc	112.4 a	0.19 a	21.1 c	2.68 defg	238.3 ab	2.64 ab	6.15 ab	0.46 abc	8.66 a
Krymsk® 1	3.64 a	136.3 a	0.20 a	28.1 ab	2.87 bcdefg	228.3 ab	1.82 c	5.59 ab	0.28 e	9.97 a
Pacer-01.36	3.39 abc	113.3 a	0.20 a	22.1 bc	2.94 abcdef	224.4 ab	2.70 ab	5.96 ab	0.42 cd	9.68 a
Padac-04.03	3.34 abc	125.5 a	0.20 a	23.6 abc	2.89 bcdefg	211.4 b	2.79 ab	5.26 ab	0.39 cde	8.68 a
Padac-150	3.44 abc	128.6 a	0.22 a	27.3 abc	3.02 abcde	243.4 ab	2.25 bc	5.36 ab	0.36 de	9.56 a
Penta	3.34 bc	120.9 a	0.22 a	25.5 abc	3.29 abcd	241.4 ab	2.77 ab	5.89 ab	0.38 cde	8.96 a
Polluce	3.41 abc	129.3 a	0.23 a	26.6 abc	3.42 ab	224.4 ab	2.85 ab	5.89 ab	0.44 cd	9.12 a
PS	3.36 abc	148.5 a	0.23 a	28.9 a	3.45 ab	256.4 ab	2.26 bc	6.74 a	0.45 bcd	10.46 a
Rootpac® 20	3.64 a	136.4 a	0.23 a	26.4 abc	3.01 abcde	238.0 ab	2.29 bc	5.40 ab	0.36 de	9.35 a
Rootpac® 40	3.15 c	117.3 a	0.21 a	24.6 abc	3.28 abcde	221.9 ab	2.92 ab	6.06 ab	0.43 cd	8.96 a
Rootpac® 70	3.27 bc	136.0 a	0.21 a	23.9 abc	3.51 a	228.3 ab	2.87 ab	6.08 ab	0.56 a	9.66 a
Tetra	3.22 bc	132.5 a	0.22 a	28.4 ab	3.52 a	258.4 ab	2.48 bc	5.66 ab	0.35 de	9.62 a
$P \leq 0.05$	***	*ns*	*ns*	***	***	**	***	**	***	*ns*
Reference values[a]	*2.0-3.5*	*70-115*	*0.12-*	*15-25*	*1.8-3.0*	*150-200*	*1.5-3.0*	*10-20*	*0.3-0.65*	*4-8*

[a] According to Villar and Arán (2008).

Data were evaluated by one-way analysis of variance (ANOVA); *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns, not significant. Means within a column followed by different letters denotes significant differences among treatments (Tukey's honestly significant difference, P≤ 0.05).

Table 6. Leaf and fruit micro elements of ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks at 120 days after full bloom. Mean values of 2015 and 2016 expressed as mg kg^{-1}.

Rootstock	Fe		Mn		Zn		B		Cu	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
AD-105	125.0 a	12.1 abc	29.9 ab	3.81 ab	37.4 abc	16.6 a	30.2 bc	1.83 fg	9.9 abcd	12.2 a
Adesoto® 101	99.4 a	9.5 bc	27.1 abc	3.55 ab	38.8 ab	10.0 b	29.6 bc	1.81 fg	9.8 abcd	11.6 a
Cadaman® Avimag	106.0 a	10.7 abc	21.3 bc	2.97 abc	34.0 abcdef	9.0 b	33.9 abc	2.64 bc	8.4 e	6.1 e
Castore	100.9 a	14.3 abc	22.0 abc	3.44 abc	33.4 abcdef	11.1 b	32.1 bc	1.88 efg	9.2 bcde	8.6 abcde
Controller 5	106.3 a	10.0 bc	23.4 abc	2.20 c	28.5 def	8.1 b	27.9 c	1.96 defg	8.4 e	6.6 de
Controller 9	105.1 a	8.8 bc	22.1 abc	2.35 c	28.6 def	7.9 b	34.4 abc	2.65 bc	8.4 e	6.2 de
INRA® GF-677	109.8 a	10.1 abc	21.9 abc	2.88 abc	36.5 abcd	10.0 b	31.5 bc	2.18 cdefg	9.4 abcde	8.3 abcde
IRTA-1	106.5 a	9.3 bc	21.8 bc	3.45 abc	33.4 abcdef	10.5 b	27.9 c	2.78 bc	9.9 abcd	10.0 abcde
Ishtara®	98.8 a	11.6 abc	23.3 abc	2.74 bc	35.1 abcde	9.9 b	35.0 abc	2.67 bc	8.6 de	7.8 bcde
Krymsk® 1	108.9 a	12.1 abc	24.5 abc	2.64 bc	37.6 bc	9.9 b	35.5 ab	1.90 efg	9.6 abcde	7.9 abcde
Pacer-01.36	104.3 a	9.1 bc	22.5 abc	3.25 abc	28.8 def	10.8 b	36.8 ab	2.80 bc	8.9 cde	7.6 bcde
Padac-04.03	96.2 a	12.8 abc	25.6 abc	3.16 abc	40.9 a	10.4 b	34.0 abc	2.78 bc	9.6 abcde	9.1 abcde
Padac-150	102.6 a	10.7 abc	29.4 ab	3.55 abc	39.6 ab	10.0 b	29.8 bc	1.63 g	9.6 abcde	8.8 abcde
Penta	98.8 a	13.6 abc	32.0 a	4.06 a	37.8 ab	11.3 b	35.8 ab	2.50 bcde	9.8 abcd	10.8 abcd
Polluce	119.9 a	14.4 abc	20.0 bc	2.86 abc	32.1 bcdef	9.4 b	33.6 abc	2.25 bcdefg	10.3 ab	9.3 abcde
PS	99.1 a	10.3 abc	20.8 bc	3.05 abc	29.4 cdef	10.5 b	39.9 a	2.84 ab	10.0 abc	8.0 abcde
Rootpac® 20	131.5 a	8.4 c	25.6 abc	3.05 abc	37.6 ab	9.5 b	36.1 ab	2.53 bcd	10.5 ab	9.9 abcde
Rootpac® 40	97.6 a	15.3 ab	21.0 bc	4.14 a	26.6 f	12.5 ab	35.4 abc	2.58 bc	9.3 bcde	10.9 abcd
Rootpac® 70	107.6 a	14.4 abc	17.7 c	2.75 bc	27.4 ef	10.0 b	40.4 a	3.44 a	9.6 abcde	9.5 abcde
Tetra	115.3 a	16.5 a	26.0 abc	3.73 ab	34.5 abcdef	10.8 b	34.8 abc	2.35 bcdef	10.6 a	11.1 abc
P ≤0.05	*ns*	***	***	***	***	**	***	***	***	***
Reference values	*60-250*	*n.d.*	*20-160*	*n.d.*	*20-50*	*n.d.*	*10-50*	*n.d.*	*4-16*	*n.d.*

[a] According to Villar and Arán (2008)

Data were evaluated by one-way analysis of variance (ANOVA); *** P ≤ 0.001; ** P ≤ 0.01; *P ≤ 0.05; ns, not significant. Means within a column followed by different letters denotes significant differences among treatments (Tukey's honestly significant difference, P≤ 0.05).

Table 7. Effect of rootstock on leaf chlorophyll concentration measured as SPAD values over time.

Rootstock	17th April				30th April				15th May				30th May				12th June				27th June			
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
AD-105	40	39	35	40	37	39	34	40	37	38	38	33	37	36	35	36	33	36	35	39	33	29	26	37
Adesoto® 101	37	37	41	40	36	36	40	38	37	37	33	31	34	34	38	35	36	37	37	33	34	35	37	34
Cadaman® Avimag	39	39	39	37	40	40	41	38	39	39	30	37	38	32	32	34	36	34	33	33	34	32	29	31
Castore	40	40	41	40	41	39	38	40	39	39	33	35	35	35	38	33	34	31	34	32	25	30	31	23
Controller 5	42	35	40	41	42	37	39	41	39	36	30	33	33	32	32	33	34	32	33	29	31	25	27	30
Controller 9	39	38	41	29	37	39	38	32	31	33	29	25	32	33	34	23	28	28	35	19	26	29	31	14
INRA® GF-677	40	40	38	42	41	42	37	40	37	39	32	33	40	36	37	36	41	38	37	31	33	34	35	28
IRTA-1	39	42	33	40	38	39	37	37	37	34	31	29	30	26	32	32	31	30	30	31	21	23	25	30
Ishtara®	41	38	42	40	43	37	43	41	38	31	33	30	31	26	35	26	32	26	31	22	27	15	26	17
Krymsk® 1	37	37	35	33	38	37	37	38	31	26	32	29	29	27	29	27	22	24	25	21	9	15	10	14
Pacer-01.36	44	38	43	36	42	38	47	37	38	36	33	39	35	33	35	32	25	32	28	31	20	28	17	28
Padac-04.03	44	37	42	44	38	35	42	40	34	35	34	32	30	35	34	35	30	35	36	37	21	33	32	35
Padac-150	40	37	40	40	42	38	41	43	38	37	38	39	32	34	33	36	35	36	33	31	34	34	33	32
Penta	39	43	39	37	35	35	37	42	34	36	28	39	27	31	28	35	29	34	30	33	21	34	30	32
Polluce	38	28	38	38	38	31	37	31	36	27	31	36	33	24	33	33	30	23	30	29	25	19	31	28
PS	41	34	40	26	32	36	36	33	24	34	23	25	21	23	24	21	19	23	18	18	8	9	10	11
Rootpac® 20	40	36	38	34	42	37	40	36	33	33	27	34	31	30	31	31	24	26	33	26	21	18	25	24
Rootpac® 40	37	37	38	28	35	37	38	37	34	37	33	34	29	34	32	30	30	33	32	30	26	31	22	28
Rootpac® 70	38	35	35	17	35	40	38	25	36	30	30	21	35	31	32	18	35	34	32	16	25	26	25	17
Tetra	37	39	36	41	36	40	38	40	36	40	36	36	32	32	32	34	34	36	29	34	26	32	21	32

Table 8. Yield, fruit weight and fruit quality parameters of the ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks in 2019. Grey bars represent variable value.

Rootstock	Yield (kg tree^{-1})	Fruit weight (g)	FF (N)	SSC (ºBrix)	TA (g L^{-1})
AD-105	29.9 ab	138.9 b	40.2 ab	14.1 ab	6.2 a
Adesoto® 101	28.7 ab	153.5 ab	38.6 ab	14.7 ab	5.4 a
Cadaman® Avimag	43.7 a	149.4 ab	45.4 a	11.7 ab	6.1 a
Castore	35.9 ab	148.2 ab	43.5 ab	13.1 ab	5.5 a
Controller 5	30.9 ab	140.4 ab	40.3 ab	13.5 ab	4.3 a
Controller 9	21.9 ab	145.8 ab	37.1 ab	12.5 ab	4.8 a
INRA® GF-677	39.7 a	144.3 ab	43.8 a	13.3 ab	5.4 a
IRTA-1	28.4 ab	148.0 ab	41.3 ab	13.5 ab	5.3 a
Ishtara®	29.2 ab	144.3 ab	44.7 a	12.6 ab	4.7 a
Krymsk® 1	12.7 b	136.2 b	34.8 b	15.9 a	3.9 a
Pacer-01.36	35.9 ab	150.3 ab	43.1 ab	13.4 ab	5.4 a
Padac-04.03	38.2 ab	149.2 ab	40.9 ab	13.8 ab	5.0 a
Padac-150	31.3 ab	148.6 ab	40.5 ab	14.9 ab	5.4 a
Penta	35.4 ab	144.8 ab	40.7 ab	12.8 ab	5.8 a
Polluce	30.3 ab	142.9 ab	43.1 ab	13.1 ab	5.5 a
PS	20.3 ab	134.5 b	44.7 a	12.6 ab	5.5 a
Rootpac® 20	28.3 ab	142.7 ab	41.7 ab	13.4 ab	5.3 a
Rootpac® 40	24.3 ab	160.1 a	38.5 ab	11.8 ab	5.6 a
Rootpac® 70	26.5 ab	147.8 ab	43.7 ab	11.3 b	6.4 a
Tetra	38.0 ab	142.9 ab	42.4 ab	13.2 ab	5.7 a
$P \leq 0.05$	*	*	**	*	*ns*

Data were evaluated by one-way analysis of variance (ANOVA); *** $P \leq 0.001$; ** $P \leq 0.01$; *$P \leq 0.05$; ns, not significant. Means within a column followed by different letters denotes significant differences among treatments (Tukey's honestly significant difference, $P \leq 0.05$).

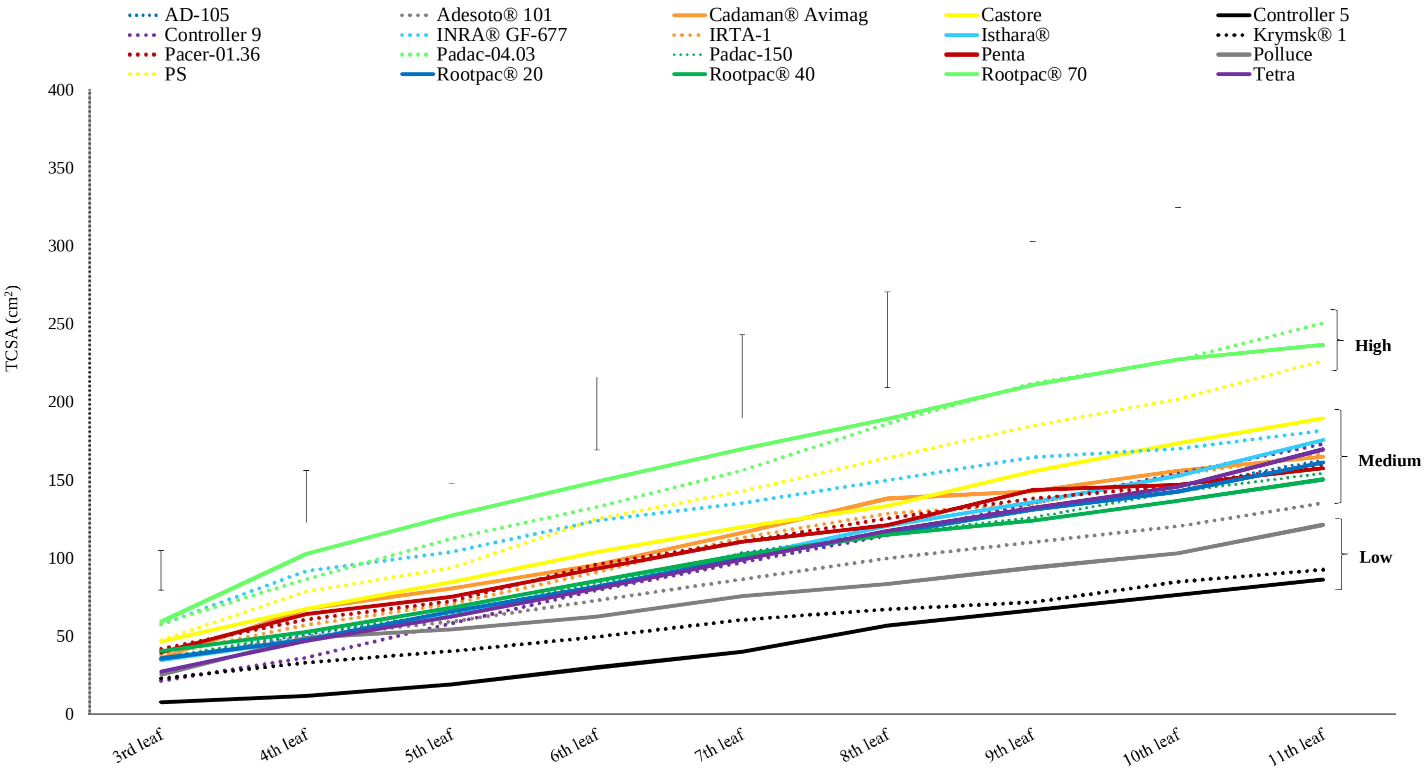


Figure 1. Effect of rootstock on trunk cross-sectional area (TCSA) of ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks during 11 years of study. Vertical lines indicate LSD ($P \leq 0.05$).

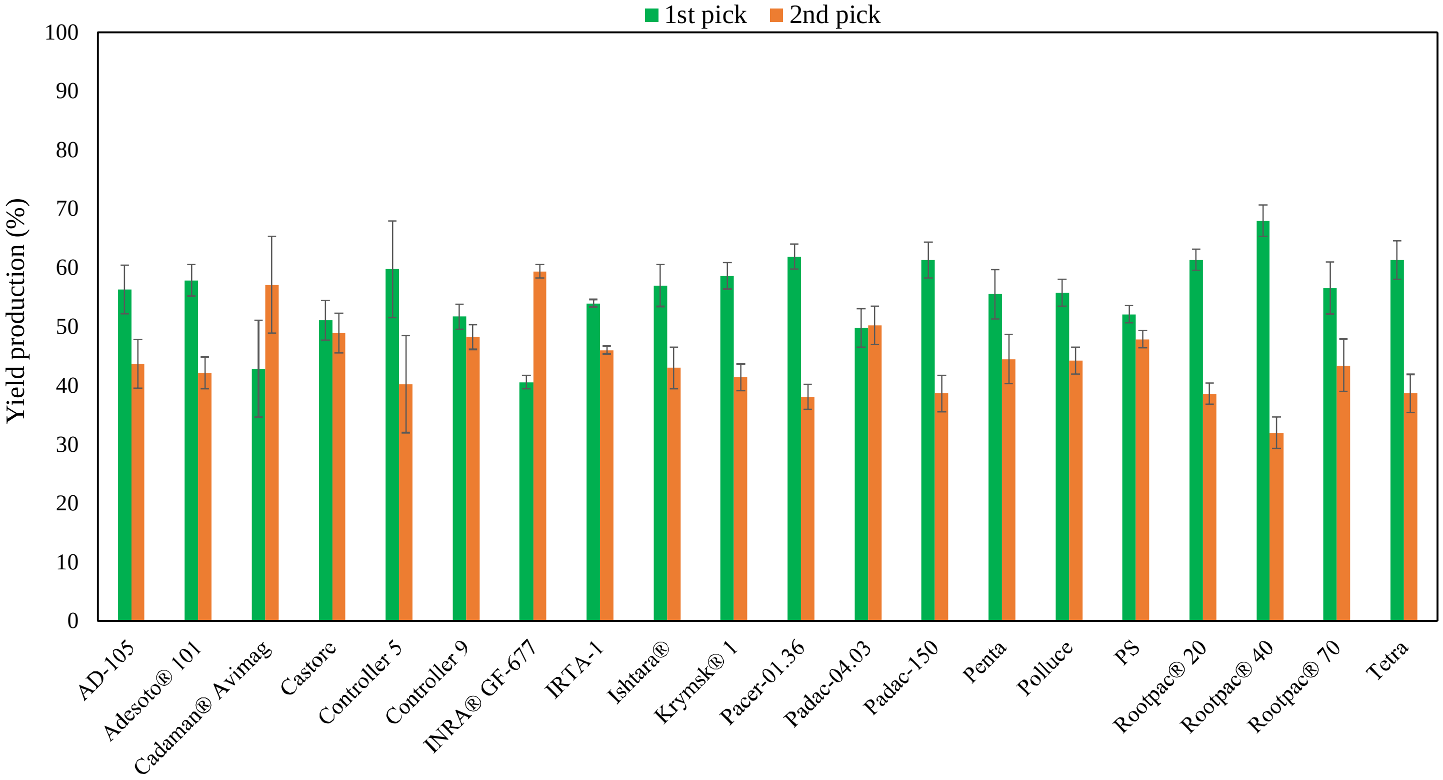
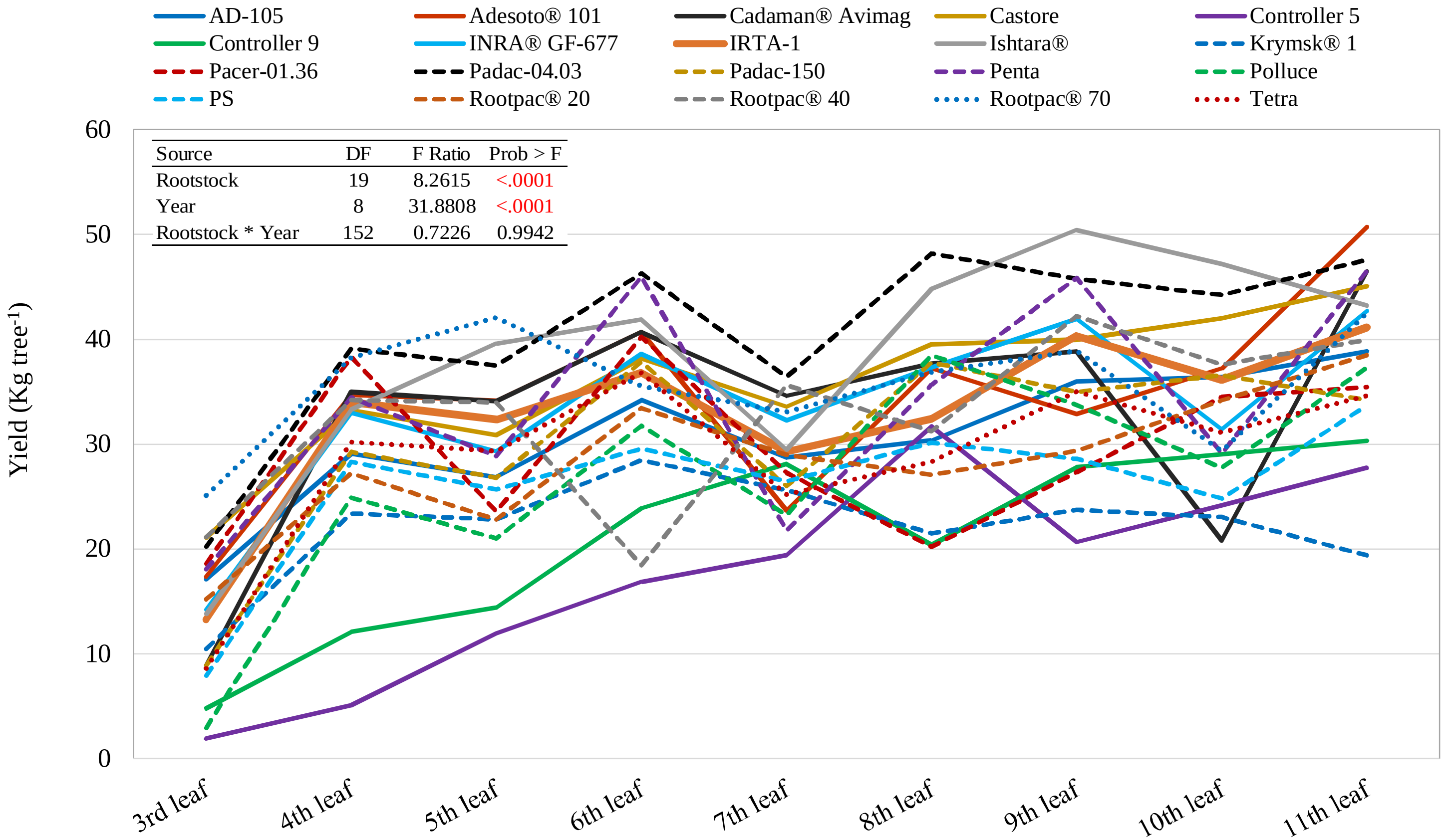


Figure 2. Mean yield (from 3rd leaf to 11th leaf) percentage for each harvest (1st and 2nd pick) of 'Big Top' nectarine cultivar grafted on 20 *Prunus* rootstocks.



Source	DF	F Ratio	Prob > F
Rootstock	19	8.2615	<.0001
Year	8	31.8808	<.0001
Rootstock * Year	152	0.7226	0.9942

Figure 3. Annual yields (kg tree^{-1}) of ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks.

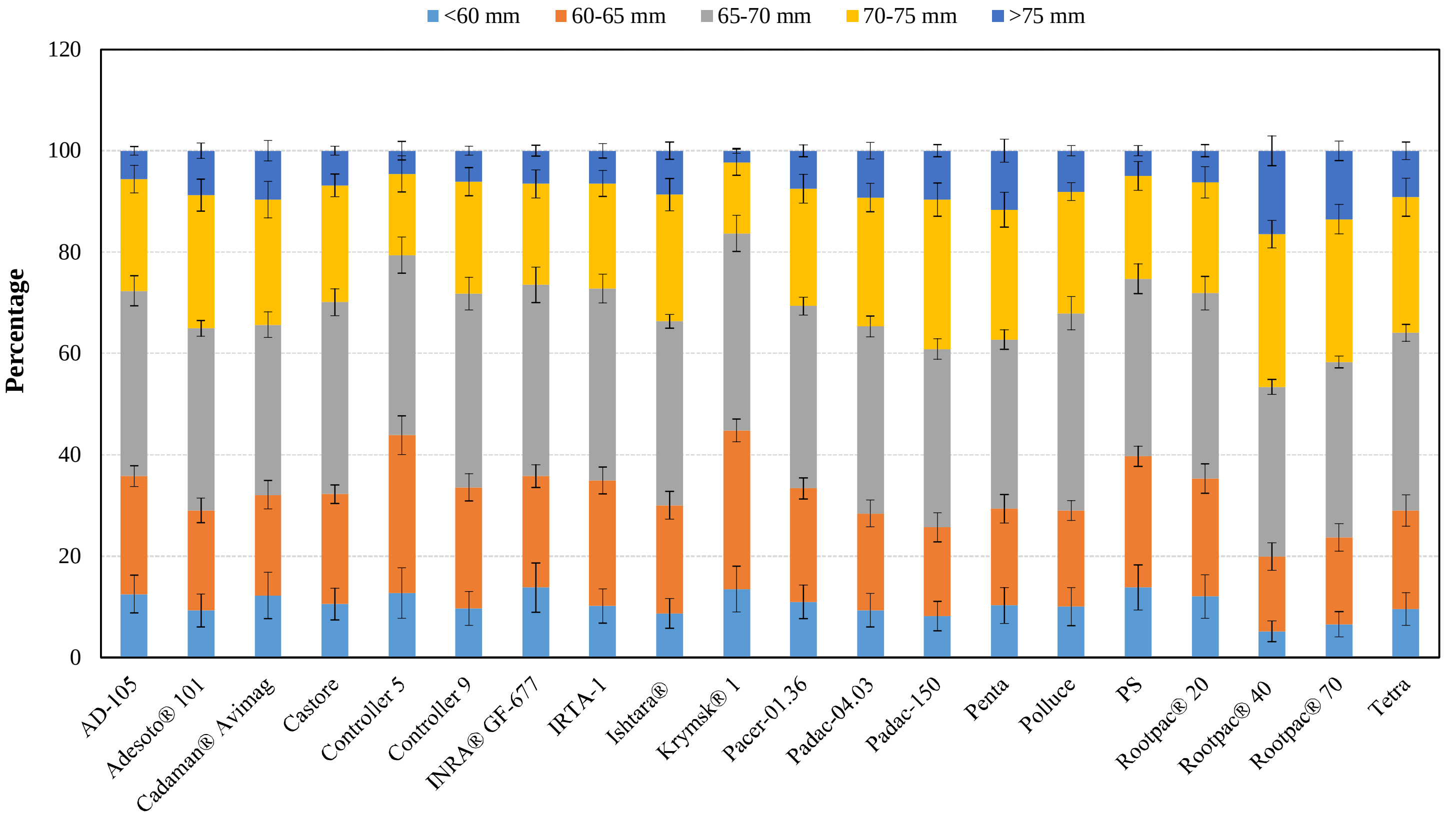


Figure 4. Mean fruit size distribution (from 3rd leaf to 11th leaf) of fruits from ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks. Vertical bars indicate the standard error.

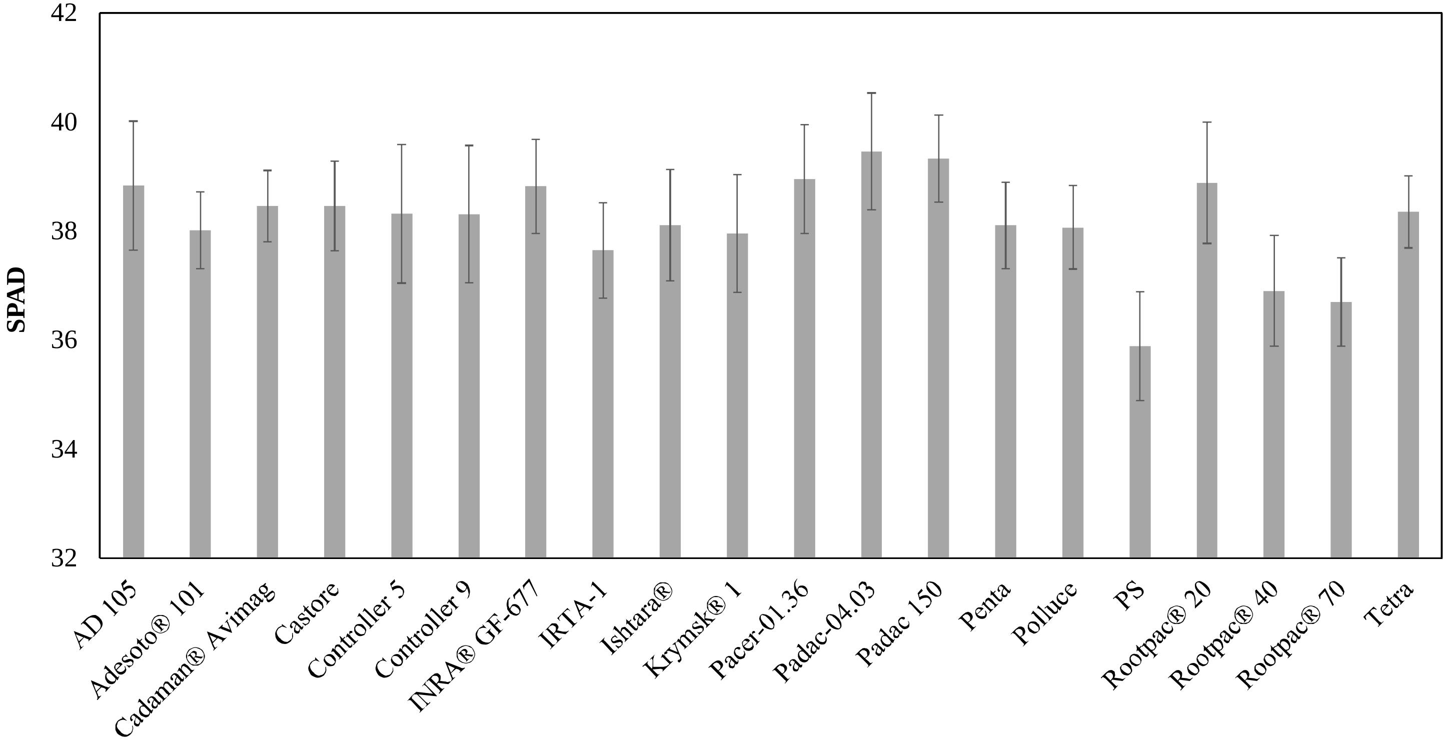


Figure 5. Effect of rootstock on leaf chlorophyll concentration (mean values of 2015 and 2016 seasons) measured as SPAD values of 'Big Top' nectarine cultivar. Vertical bars indicate the standard error.

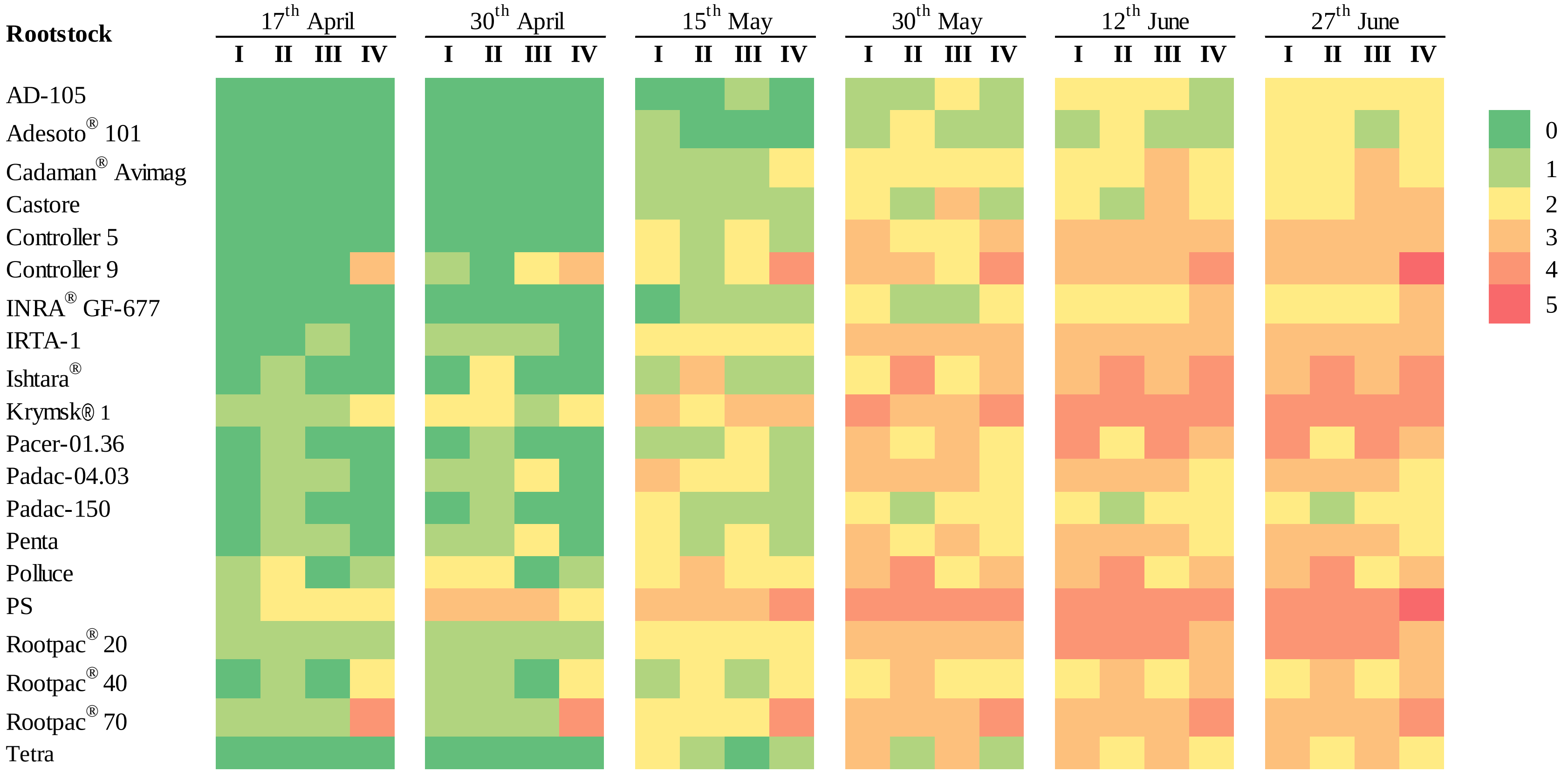


Figure 6. Evolution of chlorosis symptoms over time of ‘Big Top’ nectarine cultivar grafted on 20 *Prunus* rootstocks in 2019, where 0: no symptoms of chlorosis and 5: maximum degree of chlorosis.

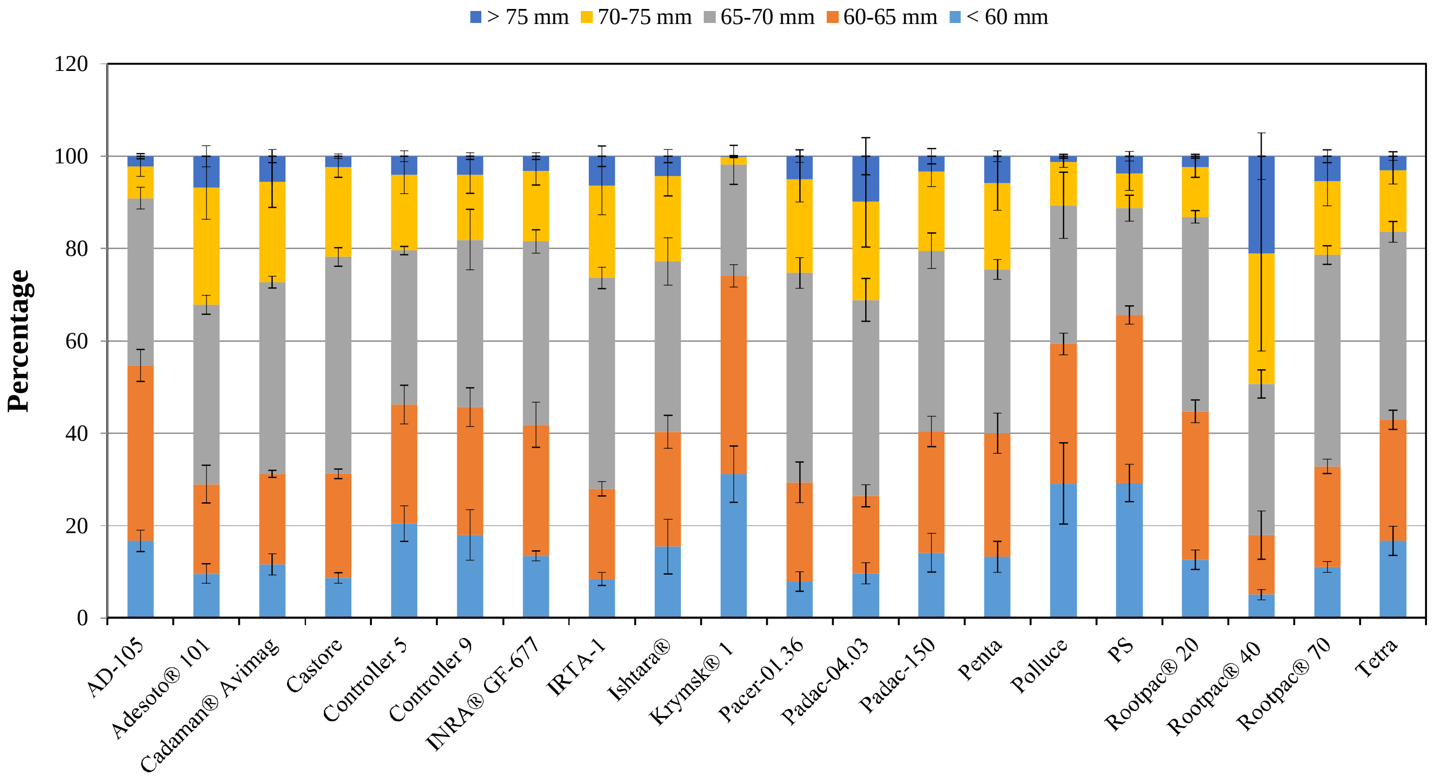


Figure 7. Fruit size distribution of fruits from ‘Big Top’ on 20 *Prunus* rootstocks in 2019. Vertical bars indicated the standard error.