Rootstock affects quality and phytochemical composition of 'Big Top' nectarine fruits grown under hot climatic conditions

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Abstract

This study aimed to evaluate the stability of 'Big Top' nectarine fruit quality (fruit weight, fruit mineral elements and fruit phytochemical composition such as soluble solids content, titratable acidity, individual sugars, individual organic acids, total ascorbic acid content, total phenolics content, and antioxidant capacity) when grafted on 20 *Prunus* rootstocks over two consecutive seasons. For most of the evaluated traits, rootstock was the main source of variability, whereas for Mg, malic and citric acids, and glucose most of the variability was observed among years. Similarly, the interaction *year* × *rootstock* was not significant for most traits (14 out of 21), hence highlighting that most rootstocks responded in a similar manner to changes in the weather conditions. Thus said, some important micro- and macro-nutrients such as Ca, Mg, Fe, Zn, together with taste- (fructose, glucose and sucrose) or health-related (antioxidant capacity) compounds showed a differential influence of the rootstock depending on the year conditions and thereby suggested that climatic conditions can be a limiting factor in the choice of rootstocks for a given nectarine cultivar. Overall, the results from this study indicated that the cherry-plum hybrid Krymsk-1 and the peach-plum hybrid PS rootstocks are the most suitable rootstocks for 'Big Top' under the conditions investigated herein. Both rootstocks induced high values on sugar profile, ascorbic acid, antioxidant activity, and TPC of 'Big Top' nectarine being relatively stable regardless of the weather conditions. Finally, the rootstocks IRTA-1 and Rootpac-20 also induced good fruit quality and phytochemical properties to 'Big Top' fruit.

Keywords: ascorbic acid, individual sugars, organic acids, total phenolics content, mineral elements

Introduction

Fruit quality is a complex concept encompassing sensory properties (appearance, texture, taste, and aroma), nutritive value, mechanical properties, safety, and defects (Crisosto and Costa, 2008). All together these attributes give the fruit a degree of excellence and an economic value (Abbott, 1999). A key step for the commercial expansion of nectarine production is undoubtedly the promotion and maintenance of the highest possible standards of the cultivar scion fruit quality. This involves the accurate evaluation of cultivar and rootstock (genotype) responses to pre-harvest factors such as growth conditions and management strategies, as well as the identification of their best combinations (Giorgi et al., 2005). Concomitant, a better understanding of these factors and the maintenance of quality through proper postharvest handling (Crisosto et al., 1997; Kyriacou and Rouphael, 2017) may counteract the current decreasing trends in consumption of nectarines experienced both on the EU and USA (Iglesias and Echeverría, 2009; Iglesias and Echeverría, *in press*). Currently, fruit consumption choices are no longer based purely on taste and personal preference, but rather on purchasing multifunctional foods. In fact, fruit quality is increasingly judged by consumers by both nutritional (minerals, vitamins, dietary fiber) and health-promoting (antioxidants) properties (Crisosto and Costa, 2008; Reig et al., 2013, 2015, 2016).

Nectarine fruit is approximately 87% water and contains carbohydrates, organic acids, pigments, phenolics, minerals, vitamins, volatiles, antioxidants and trace amounts of proteins and lipids, which make it very attractive to consumers (Crisosto and Valero, 2008). Important sugars in nectarine are fructose, glucose, sucrose and sorbitol. Sucrose is the predominant sugar in the nectarine mesocarp at maturity, accounting for approximately 40 to 85% of the total sugars content, followed by glucose and fructose (in variable ratios), together representing approximately 10–25%, and sorbitol, accounting for less than 10% (Cirilli et al., 2016; Reig et al., 2013). Among organic acids, the most abundant acids are malic, citric and quinic, being malic the main one (Reig et al., 2013; Batista-Silva et al., 2018). Nectarine is also a good source of ascorbic acid (vitamin C), carotenoids, and phenolic compounds that act as a natural antioxidants (Byrne, 2002), yet nectarine fruit generally owns a lower total antioxidant capacity than other fruit such as strawberries, apples, or oranges. Despite this, nectarines are economically and nutritionally important because they can form a significant component of the diet during the spring and summer months (Remorini et al., 2008), when their quality and consumption is maximum.

The current market demands new *Prunus* rootstocks with better tolerance to biotic (bacterial canker, armillaria*,* crown gall, phytophtora, root-knot nematode *Meloidogyne javanica,* replant disorders) and abiotic stresses (drought, iron chlorosis, waterlogging) (Byrne et al., 2012) to which scions have limited or no resistance, together with the adaptation to various soil types, inducing a range of vigor to the cultivar and the capacity to confer improved quality and nutritional characteristics of the cultivated fruit (Iglesias, 2018; Monet and Bassi, 2008). This demand has led that since the late 90´s, *Prunus* rootstocks from worldwide breeding programs, mainly from France, Italy, Spain, Canada and USA are continuously appearing (Reighard and Loreti, 2008).

Since then, several studies have reported the influence that rootstock may have on peach and nectarine fruit quality (Caruso et al., 1996; Font i Forcada et al., 2012, 2013, 2014, 2019; Giorgi et al., 2005; Iglesias, 2018; Marra et al., 2013; Mestre et al., 2017; Minas et al., 2018; Orazem et al., 2011a, b; Scalisi et al., 2018; Reig et al., 2016; Remorini et al., 2008, 2015). Beyond rootstock, other factors such as fertilization, irrigation, canopy manipulation, weather, and climate should also be considered, as they all affect peach and nectarine fruit quality (Crisosto and Costa, 2008; Lopez et al., 2012; Font i Forcada et al., 2019; Minas et al., 2018; Scalisi et al., 2018). Weather and climate are prominent influencers of agricultural production, and the recent trends in climate change may ultimately affect not only crop yields (Kukal and Irmak, 2018) but also quality.

The study of *rootstock* × *year* interaction is crucial for the success in releasing new nectarine genotypes. In this sense, a deeper knowledge of the stability of the fruit composition in different years is important when the objective is to select the best rootstock × cultivar combination. To the best of our knowledge, there is one *Prunus* rootstocks study evaluating the influence of weather, under Mediterranean climatic conditions with hot summer and heavy and calcareous soil, on the fruit quality and nutritional properties (Font i Forcada et al., 2019). Thus, the main objective of this work was to evaluate the stability of some quality traits (fruit weight, fruit mineral elements and fruit phytochemical composition such as soluble solids content, titratable acidity, individual sugars, individual organic acids, total ascorbic acid, total phenolics content, and antioxidant capacity) of twenty rootstocks grafted on 'Big Top' nectarine over two consecutive seasons.

2. Material and Methods

2.1. Plant material, site description and experimental design

The study was carried out during two growing seasons (2015 and 2016) at one experimental orchard of IRTA-Fruitcentre (Gimenells; NE Spain; 41º 39' 18.77" N and 0º 23' 31.41" E). The mid-season reference nectarine 'Big Top' in Europe was used as the cultivar selected and is the reference among yellow flesh nectarines because of its distinctive characteristics, in particular the slow melting flesh and the sweet taste (Iglesias, 2013). Twenty rootstocks from different genetic origin were evaluated. Most of them are interespecific hybrids from different species of *Prunus* (Table 1). Cadaman and GF 677 rootstock were introduced in the trial as rootstock references. GF 677 is currently the most commonly used peach × almond hybrid rootstock in Mediterranean countries due to its tolerance to calcareous soil, lime induced iron-chlorosis, good agronomical performance and good graft compatibility with peach cultivars (Giorgi et al., 2005; Iglesias, 2018; Moreno et al., 1994).

Dormant bud trees were planted in winter 2008 on Aquic Xerofluent soil, with loam texture, pH = 8.0-8.2, low salinity (CE = 0.27-0.52 dS/m), medium to high N content (128-12 ppm), medium to low P content (21-2 ppm), low K content (425-23 ppm), 3.0-0.4% organic matter and 18-53% total calcium carbonate. Trees grew under a cold-semiarid Mediterranean climate (Bsk in the Köppen-Geiger climate classification system). Rootpac-40 was planted in winter of 2009, whereas Controller-5 and Controller-9 were planted during winter of 2010. Trees were trained to catalan vase system as reported by Iglesias (2013), spaced at 5 m x 2.6 m. The fertilizers were applied by drip irrigation, and foliar micronutrients, pesticides and insecticides were applied as necessary, following industry standards.

The experiment was established in a randomized block design with four blocks, with the basic plot consisting of three trees per rootstock-scion combination. The central tree of each basic plot was used for the study. Full bloom date, when 70-80% of total flowers were open, was recorded according to Baggiolini (1952). At commercial harvest (40 – 50 N), in each block seventy fruit per rootstock-scion combination were picked, from which 30 were used for fruit quality determinations, 30 fruit for individual acids and sugars, antioxidant capacity, total ascorbic acid and total phenols content (TPC) determinations, and 10 fruit for fruit mineral elements analysis.

2.2. Weather conditions

Meteorological data (daily minimum and maximum air temperature, degree-days, rainfall and solar radiation) from March to July, which covers the fruit growth and ripening period for each respective season, was downloaded from the meteorological station located in the experimental orchard of IRTA-Fruitcentre-Gimenells (www.ruralcat.cat). Degree-day was calculated as the difference between the daily mean temperature and a base temperature of 10 ºC (Reig et al., 2017). The area of this study has weather conditions typical of the continental Mediterranean area: with daily maximum summer temperatures of $\geq$30 ºC and accumulated rainfall between 300-400 mm throughout the year.

2.3. Determination of fruit weight, soluble solids content and titratable acidity

Fruit weight (FW), soluble solids content (SSC) and titratable acidity were measured with a Pimprenelle robotic laboratory (Setop, Cavaillon, France). FW was expressed in g, SSC was expressed in SSC percentage, which is equivalent to “degrees Brix” (ºBrix), and TA was expressed in g malic acid L^{-1}.

2.4. Determination of dry matter and fruit mineral elements

Dry matter was obtained by a gravimetry method and was expressed as percentage. All elements were obtained by inductively coupled plasma mass spectrometry (ICP-OES). Concentrations were expressed as mg 100 g^{-1} on a dry weight basis (phosphor, potassium, calcium, and magnesium), as mg kg^{-1} also on a dry weight basis (boron, iron, zinc, and manganese), and as percentage (sulfur).

2.5. Determination of total ascorbic acid

Extraction of total ascorbic acid (AsA) was done by dissolving 3 g of fresh-frozen nectarine samples into 5 mL of an aqueous solution containing 3% metaphosporic acid and 8% acetic acid (w/v). Samples were then vortexed for 1 min and further centrifuged at 24000 x g for 22 min at 4ºC. The clear supernatant was then recovered and passed through a 0.45 µm nitrocellulose syringe driven filter (Millipore Corporation, MA) and kept at -98ºC for a couple of hours prior to analysis. Total ascorbic acid determination was done, after incubation of the clear extract (950 µL) with 50 µL of TCEP-HCl (40 mM) for three hours in the dark at 4ºC, using and HPLC (Waters system) equipped with a UV/Vis detector (254nm) and a SupelcosilTM LC-18 column (25 cm x 4.6 mm x 5 µm). The mobile phase was 0.01 % (w/v) H_2SO_4 at a flow rate of 1 mL/min. Total ascorbic acid quantification was done by comparing the sample peak to that obtained with a standard (0 -50 mg/L). Results were finally expressed as mg AsA 100 g^{-1} sample.

2.6. Determination of individual organic acids and sugars

Extracts for malic and citric acid determination were prepared as described in Giné-Bordonaba and Terry (2010), with some modifications. Briefly, fresh frozen fruit tissue (2g) was added to 5 mL of HPLC-grade water and kept at room temperature (25 ºC) for 10 min prior to being centrifuged at 24,000 x g for 7 min at 20 ºC.

Glucose, fructose and sucrose were extracted from fresh-frozen material as described elsewhere (Terry et al., 2007). Briefly, 2g of sample were dissolved in 5 mL of 62.5% (v/v) aqueous methanol solvent and placed in a thermostatic bath at 55 ºC for 15 min, mixing the solution with a vortex every 5 min to prevent layering. Then, samples were centrifuged as described above.

The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of malate (L-malate dehydrogenase), citrate (citrate lyase/malate dehydrogenase), and fructose, glucose and sucrose (β-fructosidase/hexokinase/phosphoglucose isomerase) as described by Giné-Bordonaba et al. (2017) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions. Organic acids (malic and citric acid) and sugars (glucose, fructose and sucrose) were expressed in mg g^{-1} FW.

2.7. Determination of antioxidant capacity and total phenolics content

Antioxidant capacity and total phenolic concentrations of nectarine fruit were quantified from fresh-frozen material as described earlier (Giné-Bordonaba and Terry, 2008) by mixing 3 g of sample with 1.5 mL of 79.5% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water. Sample extraction was held at 25 ºC with constant shaking for 2h and mixing the samples every 15 min (Giné-Bordonaba and Terry, 2016). Finally, samples were filtered through a 0.2 µm syringe driven filter unit (Millipore Corporation, MA) and the clear extract analysed. From the same extract, total phenolic compounds (mg gallic acid equivalents (GAE) g^{-1} FW) were measured by means of the Folin-Ciocalteu method and total antioxidant capacity (mg Fe^{2+} per g^{-1} FW) measured by the Ferric Reducing Antioxidant Power (FRAP) assay as described in recent works (Giné-Bordonaba and Terry, 2016).

2.8. Statistical analysis

On the comparative study of the two harvest seasons, data were subjected to a two-way ANOVA analysis, in order to examine year (Y), rootstock (R), and Y × R interaction. The total variability of each parameter was estimated using the total sum squares of two-way ANOVA results. Additionally, the variability expressed as percentage of the total sum of squares for year, rootstock, and the interaction between both was calculated. Mean separation was assessed by LSD test and Tukey HSD test with a *P* value of 0.05 using the JMP statistical software package (Version 13, SAS Institute Inc., Cary, North Carolina). Spearman's rank correlation matrix ($P < 0.05$) was done using the R coreplot package.

3. Results

Despite Rootpac-40 was planted one year later, and Controller-5 and Controller-9 two years later, fruit harvest in 2015 and 2016 from each rootstock-scion combination were from mature trees, considering 5 or more years-old tree as mature tree. Therefore, in this study we do not consider the age of tree as a factor affecting the phytochemical fruit profile of the different rootstock-cultivar combinations evaluated herein.

3.1. Weather conditions

Full bloom and harvest dates are shown in Table 2. The period between full bloom and harvest dates (fruit development period) was shorter in 2015 than in 2016 (Table 2) and was characterized by higher accumulated degree-days and mean daily temperature, but lower accumulated rainfall and solar radiation. As an average, the evaluated trees bloomed 5-7 days earlier and fruit were harvested 8 days later in 2016 than in 2015.

Minimum and maximum mean temperatures were higher in 2015 (1.5 and 2.5 ºC, respectively) if compared to those from 2016. However, during the same period, the accumulated rainfall and solar radiation were higher in 2016 than 2015 (Table 2, Supplemental Figure 1).

3.2. Stability on fruit weight, soluble solids content, and titratable acidity

The total variance observed for each trait evaluated and the factor that mostly caused the variability is shown in Fig. 1. Within the fruit quality traits evaluated, fruit weight (FW) owned the highest reported variability, mainly attributed to the rootstock. Similarly, the variability associated to the total soluble solids content (SSC) and titratable acidity (TA) was also mainly caused by the rootstock. Accordingly, and as depicted in Table 3, the analysis of variance (ANOVA, $p \leq 0.05$) showed that year and rootstock, but not the interaction of both factors, significantly affected all these traits (FW, SSC, and TA). In 2015, fruit were, in general, 10 g heavier and sweeter than those from 2016 (Table 4). Considering the differences among rootstocks for these quality traits, 'Big Top' grafted on Rootpac-40 and Padac-150 rootstocks consistently produced bigger fruit, although they were not significantly different from those produced by the rest of the rootstocks except for Controller-5, which own the smallest fruit (Table 4, Supplemental Figure 2). Krymsk-1 induced sweeter fruit, followed by IRTA-1, Controller-5, Pacer-01.36 and PS, whereas those fruit from Tetra, Rootpac-70, Padac 150 and AD 105 had high TA values, and those from Controller-5 and Controller-9 had low TA values.

Significant correlations were found between most fruit quality traits (Figure 3). Fruit weight was moderate to high negatively correlated with SSC and positively correlated with TA. In addition, SSC was negatively correlated with degree days (DD).

3.3. Stability on fruit dry matter and mineral composition

The variability observed in dry matter, and macro and micro minerals are shown in Fig. 1. The variability associated to potassium (K) concentrations was particularly high. In detail, rootstock was the main source of variability (around 50%) for the K content. Rootstock was also the main source of variability for phosphor (P), calcium (Ca), boron (B), iron (Fe), zinc (Zn), manganese (Mn), and sulfur (S) concentrations, while year was the most important factor for magnesium (Mg).

ANOVA analysis reported that year had no significant effect on the microelements B, Fe, and Zn (Table 3). The *year* × *rootstock* interaction had significant effect on the macro elements Ca and Mg, and the microelements Fe, and Zn. Except for B, and Fe concentrations, which were similar on both years, 'Big Top' fruit from 2015 had less dry matter content and lower macro and microelements concentration in the flesh than the following year (Table 4). K was the main macroelement found in 'Big Top' fruit, whereas in terms of microelements, Fe represented the principal element in all the analyzed samples (Table 4). In general, 'Big Top' fruit from PS rootstock had the higher dry matter content, followed by those from Padac-150 and Rootpac-20, whereas those from Rootpac-40 had the lowest value (Table 4, Supplemental Figure 2). Concerning the macro elements (P, K, Ca, and Mg), those fruit from PS rootstock had the highest mean values of all of them. ‘Big Top’ fruit from AD-105 and Tetra had also high K mean values, whereas IRTA-1 had also high mean values of Ca. The lowest values for P, K, and Ca were for Isthara, Padac-04.03, Adesoto, respectively, whereas the lowest Mg values were for Isthara, Padac-04.03 and Cadaman. Concerning the micro mineral elements, fruit from Rootpac-70 had high B values, followed by those from PS, whereas Padac-150 had the lowest one. Tetra induced fruit with high Fe concentration, whereas those from Controller-9 and Rootpac-20 had the lowest value. The highest Zn concentration was observed on AD-105, whereas low value was observed on Controller-5 and Cadaman, respectively. Finally, the highest Mn values were for fruit from Rootpac-40, followed by Penta and AD-105, whereas the lowest ones were for fruit from Controller-5 and Controller-9.

Based on the *year* × *rootstock* interaction significant effect, Castore and PS rootstocks in 2015 induced higher Ca concentration in fruit (Figure 3a), whereas the lowest values were observed on those from Adesoto, Cadaman, Padac-0403, Padac-150 and Tetra in 2016. Regarding Mg concentration in fruit flesh, Krymsk-1, PS, Rootpac-70 and Tetra had the highest values, all of them in 2015, whereas, the lowest Mg values were for Castore, Controller-9, Isthara, Padac-04.03, Penta, Polluce, Rootpac-40, Rootpac-70 and Tetra, all of them in 2016 (Fig. 3b). Among the microelements (Fe and Zn), ‘Big Top’ fruit from Polluce, Rootpac-40 and Rootpac-70, all in 2015, together with those from Tetra (2016) had the highest Fe values, whereas those fruit from Controller-9 in 2016 had the lowest value (Figure 4a). Finally, AD-105 in 2015 induced the highest Zn value, whereas Controller-5 in 2016 the lowest one (Fig. 5b).

Some interesting correlations were found among ‘Big Top’ fruit minerals (Fig. 2). Dry matter content was moderate and positively correlated with K. P was negatively correlated with B. K was moderate correlated with P, Zn, and S. Mg was moderate to high positively correlated with dry matter, P, K, and Ca. Mn was highly correlated with Zn, and S, and S was highly correlated with Zn. In addition, fruit quality traits were significantly correlated with some elemental minerals. FW was negatively correlated with dry matter and positively correlated with Fe, Mn and S, whereas SSC was positively correlated with dry matter and Mg. Finally, TA was positively correlated with K, Zn, Mn and S.

3.4. Stability on total ascorbic acid content, individual organic acids and sugars

Overall, the content of individual acids showed little variability (Figure 1) across rootstocks or years if compared to the other parameter investigated herein. For instance, rootstock was the main source of variability for ascorbic acid, whereas the year was the most important source of variation for the organic acids, malic and citric. Among the individual sugars, the highest variability appeared in the sucrose content, being the interaction *year* × *rootstock* the main source of variability. A major effect of rootstock was reported for fructose, while in the glucose concentration the year seemed to be the main source of variation.

As shown in Table 3, analysis of variance (ANOVA, $p \leq 0.05$) showed that year had no effect on fructose content, whereas rootstock had significant effect on all sugars and organic acids, including ascorbate, except for citric acid. The *year* × *rootstock* interaction was only significant for the individual sugars (fructose, glucose, sucrose). Overall, 'Big Top' fruit had higher glucose, but lower ascorbic, citric and malic acids content, and sucrose content in 2015 compared to 2016 (Table 4).

On average, slightly significant differences were found among rootstocks for ascorbic acid. The highest ascorbic acid values were on PS fruit, although it did not significantly differ from Krymsk-1, Controller-5, Padac-150, Rootpac-20, Polluce and AD-105 (Table 4, Supplemental Figure 2). Rootpac-40 fruit had the lowest ascorbate content, yet it did not differ significantly from the rest of the rootstocks except for PS. Malic acid and citric were two of the three dominant organic acids in the evaluated nectarine fruit accounting in average for 62 and 38% of the total acid content, respectively. Regarding malic acid, the highest level was found in the 'Big Top' fruit on PS rootstock (Table 4, Supplemental Figure 2). Fruit from both Controllers rootstocks together with Cadaman had the lowest values, but they did not differ statistically from the rest of the rootstocks, except for PS.

Sucrose was the dominant sugar, glucose was the second most abundant sugar, and fructose was the minor sugar in nectarine fruit (Table 4) accounting, in average, for 80, 11 and 9%, respectively, of the total sugars evaluated. Regarding fructose content, on average, fruit from PS rootstock had the highest content, whereas those from Penta had the lowest value, followed by Rootpac-70 (Table 4, Supplemental Figure 2). In particular, 'Big Top' fruit from PS in 2015 had the highest value, where those from Rootpac-70 in 2016 had the lowest value (Figure 5a). The glucose content of the different rootstocks was highest for Krymsk-1, whereas Polluce induced the lowest value. In contrast, according to the significant effect of the interaction, fruit from PS in 2015 had the highest value and those from Padac-04.03 had the lowest one (Figure 5b). For sucrose content, on average fruit from IRTA-1 owned the highest value, whereas fruit from Rootpac-70 had the lowest value.

Moderate positive correlations were found between ascorbic acid and malic acid, fructose and glucose, and between glucose and sucrose (Fig. 2). Ascorbic acid, the organic acids (malic and citric) and the individual sugars (fructose, glucose and sucrose) had also significant correlations with certain fruit quality traits and dry matter and macro- and microelements. In fact, it is interesting to mention those between ascorbic acid and dry matter, malic acid and N, fructose and FW, among others. Finally, citric acid was moderate and positively correlated with mean daily temperature and negatively correlated with accumulated rainfall thereby partly explaining the high influence of the year on citric acid content described earlier.

3.5. Stability on antioxidant capacity and total phenols content

The variability observed in the antioxidant capacity and the total phenols content (TPC) was low (Fig. 1). Rootstock and the *year* × *rootstock* interaction were the main sources of variability for both traits, respectively. Despite this, ANOVA analysis reported significant effect of year, rootstock, and their interaction on the fruit antioxidant capacity and TPC (Table 3).

‘Big Top’ fruit had, in general, higher antioxidant capacity, but lower TPC in 2015 compared to 2016 (Table 4). Among rootstocks, on average, the highest fruit total phenolic content (TPC) was found in Krymsk-1, followed by Padac-150, PS, and Rootpac-20, whereas the lowest values were found in Cadaman, Rootpac-40, Tetra, Polluce and IRTA-1 (Table 4, Supplemental Figure 2). The highest antioxidant capacity values were for Krymsk-1 and PS (Table 4, Supplemental Figure 2), in particular in 2015 (Figure 6). As expected, moderate to high positive correlation was found between TPC and antioxidant capacity (Fig. 2). Both TPC and antioxidant activity were also significantly and positive correlated with SSC, dry matter, ascorbic acid, fructose and glucose, and negatively correlated with FW and accumulate degree days. Antioxidant capacity was also positive correlate with the mean daily temperature and negative correlated with the accumulated rainfall and solar radiation.

4. Discussion

Nectarine quality directly impacts its commercial value. Lurie and Crisosto (2005) reported that consumer acceptability for a product is mainly influenced by the quality of the following parameters: firmness, acidity, texture, aroma, sugars content, and antioxidant capacity. Indeed, the measurement of the total antioxidant capacity gives a good measure of the fruit nutritional value (Drogoudi and Tsipouridids, 2007).

This is the first study evaluating the stability of multiple fruit quality parameters (fruit weight, fruit mineral elements, and fruit phytochemical composition such as soluble solids content, titratable acidity, individual sugars, individual organic acids, total ascorbic acid content, total phenolics content, and antioxidant capacity) from 'Big Top' grafted on twenty *Prunus* rootstocks from different genetic background, and grown under Mediterranean climatic conditions, over two consecutive seasons. In this sense, when selecting the best rootstock-cultivar combination is crucial to evaluate and know the overall variability for most if not all main fruit quality traits, as well as to understand which fraction of this variability is explained by the year (weather conditions), the rootstock, and/or their interaction. It is also important to evaluate which climate factor (temperature, precipitation, solar radiation) is dominant in explaining the year-to-year variability.

In general, our results partially agree with available data reporting the effect of year and rootstock on FW, SSC, TA, ascorbic acid content, mineral elements, individual sugars (fructose and glucose) and organic acids (malic and citric), TPC and antioxidant capacity on different peach and nectarine cultivars (Caruso et al., 1996; Font i Forcada et al., 2013, 2014, 2019; Orazem et al., 2011a,b; Reig et al., 2016). Nonetheless, the year effect investigated in the above-mentioned studies was not correlated to any weather variable (temperature, degree growing days, rainfall, solar radiation) as done in this study.

The ANOVA table showed that for most of the evaluated traits, the interaction *year* × *rootstock* was not significant, hence highlighting that most rootstocks responded in a similar way to changes in the weather conditions. Our results also confirm the rootstock effect on specific fruit quality (SSC, TA) or biochemical traits (ascorbic acid, malic acid, and glucose) as reported by other authors (Font i Forcada et al., 2019; Orazem et al., 2011 a,b; Reig et al., 2016). The significant interaction *year × rootstock* for Ca, Mg, Fe, Zn, fructose, glucose, sucrose, and antioxidant capacity, indicated a different rootstock behavior in relation to the climatic conditions which is generally in agreement with some previous *Prunus* rootstocks studies. However, to the best of our knowledge, there are no *Prunus* studies evaluating the *year × rootstock* interaction effect on flesh mineral elements on nectarine cultivars. Font i Forcada et al. (2019) reported a significant *year × rootstock* interaction for sucrose, but not for fructose, glucose and antioxidant capacity on 'Big Top' trees grafted on several *Prunus* rootstock and grown on a heavy calcareous soil. Despite being the same cultivar analyzed, 'Big Top', differences in the weather conditions, rootstocks and soil types on the crop site are likely the causes of the observed discrepancies between studies since sugar accumulation, and translocation from source to sink is largely dependent on agroclimatic conditions (Lescourret and Gènard, 2005).

It is interesting to mention the significant negative correlation between SSC and the DD. Lopestri et al. (2014) reported that low air temperature could play a role in an overall reduction in fruit size and sugar concentration. Bonora et al. (2013) reported that riper, more exposed fruit harvested from the same trees did not have significantly higher SSC than shaded fruit. They reasoned that high temperatures prior to harvest increased fruit respiration rates resulting in less carbohydrate available for storage as soluble solids. Ours results showed that sucrose but not glucose and fructose was lower in 2015, the year with higher maximum and minimum temperatures prior to harvest.

The percentage of variability explained by year for the relatively immobile macronutrient Mg was high, despite not being correlated by any of the weather variables evaluated in this study, leading to think that other factors differing between years play a critical role in the accumulation/utilization of this compound.

Unlike soluble carbohydrates, which are imported into the fruit as photoassimilates, the majority of the organic acids present in fleshy fruit are not imported but rather synthesized *in situ*, mostly from imported sugars from glycolysis mediating starch and cell wall degradation. In fact, the accumulation of malate and citrate in fruit is seemingly a result of close interaction between metabolism and vacuolar storage and it is also controlled by several environmental factors that affect the acidity of fleshy fruit by acting on various cellular mechanisms (Etienne et al., 2013). Under our climatic conditions, malic acid was not affected by any of the weather variables evaluated in this study and hence further supporting the fact that malic acid is not an important respiratory substrate in peach fruit (Famiani et al., 2016). Our results also found that, the accumulated rainfall affected negatively the citric acid content and the antioxidant capacity of 'Big Top' fruit, whereas the mean daily temperature affected positively both traits, suggesting that lower citrate content may be the mere result of higher water uptake. Famini et al. (2016) reported that in the flesh of many fruits, the content of malate and citrate decreases during ripening, and this decrease can arise either from their metabolism or a dilution effect brought about by an increase in the volume of the fruit. Wert et al. (2007) also reported that weather in the form of precipitation may affect internal fruit quality if it occurs during the latter stages of fruit development. In agreement, our results show that despite higher accumulated rain occurred in 2016, most of the precipitations took place during early fruit development stages and hence did not affect fruit size/volume.

Cirilli et al. (2016) reported that individual sugar contents are strongly affected by seasonal variability. Under our climatic conditions, year was the main source of variability only for glucose. However, glucose concentration was not correlated with any of the weather variables evaluated in this study and suggesting that other factors may play a critical role in this trait differences between years.

Finally, knowing the rootstock which confers the best of these attributes to 'Big Top' fruit could mean an increase of its commercial value for local growers and packers. In this study, 'Big Top' trees grafted on Krymsk-1 and PS had, together with IRTA-1, Padac 150 and Rootpac-20, high to medium values on sugar profile, ascorbic acid, TPC and antioxidant activity of 'Big Top' for both years of evaluation. However, both rootstocks (Krymsk-1 and PS) showed some agronomic limiting factors as the lack of compatibility in the case of Krymsk-1 with peach and the low yield efficiency for PS.

Conclusion

The study highlighted the effect of rootstock, year, and their interaction on fruit quality and phytochemical, and suggests that climatic conditions should be a factor considered in the choice of rootstocks for a given nectarine cultivar, depending on the evaluated trait. Rootstock was the main source of variability for most quality or biochemical traits, but year was also an important factor for Mg, organic acids, and glucose. The weather conditions influenced the SSC, citric acid, TPC and the antioxidant capacity of ‘Big Top’ grafted on several *Prunus* rootstocks as per the significant *year* × *rootstock* interaction values. The cherry-plum hybrid Krymsk-1 and the peach-plum hybrid PS rootstocks are the most suitable rootstocks for ‘Big Top’ under the conditions investigated herein by inducing high values on sugar profile, ascorbic acid, antioxidant activity, and TPC of ‘Big Top’ and being relatively stable regardless of the weather conditions. The PS rootstock also induced firmer and more acids fruit. Rootstocks IRTA-1 and Rootpac-20 also induced good biochemical properties to ‘Big Top’ fruit. GF-677 rootstock did not have pronounced significant effects on the fruit quality and biochemical profile in ‘Big Top’ fruit grown in a loam soil. Finally, the results from the present study may improve our knowledge on the fruit quality and phytochemical traits of ‘Big Top’ grafted on different rootstocks and grown under loam soil conditions. Thereby, the information provided by this study will be helpful in the breeding programs and growers for further decisions.

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Table 1. List of studied rootstocks and origin.

Rootstock	Species	Breeder[a]
AD-105	*P. insititia*	CSIC, Spain
Adesoto®101	*P. insititia*	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*	Univ. Calif. Davis, (USA)
Controller-9	*P salicina x P. persica*	Univ. Calif. Davis, (USA)
INRA®GF-677	*P. amygdalus x P. persica*	INRA, France
IRTA-1	*P. amygdalus x P. persica*	IRTA, Spain
Isthara® (Ferciana)	*(P. cerasifera x P. salicina) x (P. cerasifera x P. persica)*	INRA, France
Krymsk-1 (VVA-1)	*P. tomentosa x P. cerasifera*	E.E. Krasnodar, Russian Federation
Pacer-01.36	*(P. cerasifera x P. spinosa) x (P. spinosa x P. persica)*	Agromillora, Spain
Padac-04.03	*P. cerasifera x (P. amygdalo x P. persica)*	CSIC-Agromillora, Spain
Padac-150	*P. insititia*	CSIC-Agromillora, 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 (Densipac)	*P. besseyi x P. cerasifera*	Agromillora, Spain
Rootpac®40 (Nanopac)	*P. amydalus x P. persica*	Agromillora, Spain
Rootpac®70 (Redpac)	*(P. persica x P. davidiana) x (P. amygdalus x P. persica)*	Agromillora, Spain
Tetra	*P. domestica*	CREA Rome, Italy

[a] Agromillora: private nursery, Spain; CITA: Centro de Investigación y Tecnología Agroalimentaria de Aragón; CSIC: Consejo Superior de Investigaciones Científicas; INRA: Institut National de la Recherche Agronomique.

Table 2. Bloom date, fruit development period, growing degree-days, rainfall and solar radiation of 'Big Top' grafted on 20 rootstocks grown under Mediterranean conditions.

Rootstock	Full bloom date[a]	Fruit development	Growing degree	Mean daily	Accumulated	Accumulated solar

			period[b]		days[b]		T[a] (ºC)[b]		rainfall (mm)[b]		radiation (MJ m^2)[b]	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
AD-105	21-Mar	15-Mar	103	117	1835.0	771.3	17.8	16.5	70.0	118.8	2553.3	2821.8
Adesoto® 101	20-Mar	15-Mar	104	117	1845.5	771.3	17.7	16.5	78.5	118.8	2563.2	2821.8
Cadaman® Avimag	21-Mar	16-Mar	103	116	1835.0	771.3	17.8	16.6	70.0	110.8	2553.3	2813.4
Castore	20-Mar	16-Mar	104	116	1845.5	771.3	17.7	16.6	78.5	110.8	2563.2	2813.4
Controller-5	20-Mar	11-Mar	104	121	1845.5	771.3	17.7	16.1	78.5	130.1	2563.2	2883.6
Controller-9	21-Mar	11-Mar	103	121	1835.0	771.3	17.8	16.1	70.0	130.1	2553.3	2883.6
INRA® GF-677	21-Mar	4-Mar	103	128	1835.0	771.3	17.8	15.6	70.0	134.2	2553.3	2990.9
IRTA-1	21-Mar	16-Mar	103	116	1835.0	771.3	17.8	16.6	70.0	110.8	2553.3	2813.4
Isthara® (Ferciana)	22-Mar	18-Mar	102	114	1823.8	771.3	17.9	16.7	64.0	110.8	2545.0	2783.9
Krymsk-1 (VVA1)	26-Mar	18-Mar	98	114	1783.5	771.3	18.2	16.7	48.3	110.8	2498.1	2783.9
Pacer-01.36	25-Mar	20-Mar	99	112	1791.5	769.9	18.1	16.9	52.8	105.0	2518.8	2764.0
Padac-04.03	21-Mar	12-Mar	103	120	1835.0	771.3	17.8	16.2	70.0	130.1	2553.3	2864.4
Padac-150	21-Mar	16-Mar	103	116	1835.0	771.3	17.8	16.6	70.0	110.8	2553.3	2813.4
Penta	21-Mar	11-Mar	103	121	1835.0	771.3	17.8	16.1	70.0	130.1	2553.3	2883.6
Polluce	20-Mar	15-Mar	104	117	1845.5	771.3	17.7	16.5	78.5	118.8	2563.2	2821.8
PS	21-Mar	18-Mar	103	114	1835.0	771.3	17.8	16.7	70.0	110.8	2553.3	2783.9
Rootpac® 20	21-Mar	8-Mar	103	124	1835.0	771.3	17.8	15.9	70.0	131.1	2553.3	2930.6
Rootpac® 40	21-Mar	16-Mar	103	116	1835.0	771.3	17.8	16.6	70.0	110.8	2553.3	2813.4
Rootpac® 70	21-Mar	11-Mar	103	121	1835.0	771.3	17.8	16.1	70.0	130.1	2553.3	2883.6
Tetra	21-Mar	11-Mar	103	121	1835.0	771.3	17.8	16.1	70.0	130.1	2553.3	2883.6

[a] Estimated date of 70-80% flowers open.

[b] Data from full bloom to harvest date (2015 harvest date: 2nd July, and 2016 harvest date: 10th July)

Table 3. Two-way ANOVA analysis to evaluate the effect of rootstock, year and their interaction on all quality and biochemical traits evaluated on ‘Big Top’ nectarine grafted on 20 rootstocks.

Trait	Units	Variance Analysis		
		Year (Y)	Rootstock (R)	Y x R
Fruit weight	g	**	*	ns
Soluble solids content	ºBrix	*	***	ns

Titratable acidity	g L^{-1}	***	***	ns
Dry matter	%	***	***	ns
Phosphor	mg 100 g^{-1}	***	***	ns
Potassium	mg 100 g^{-1}	***	***	ns
Calcium	mg 100 g^{-1}	***	**	*
Magnesium	mg 100 g^{-1}	***	**	***
Boron	mg kg^{-1}	ns	***	ns
Iron	mg kg^{-1}	ns	***	***
Zinc	mg kg^{-1}	ns	***	***
Manganese	mg kg^{-1}	***	***	ns
Sulfur	%	**	***	ns
Ascorbic acid	mg 100 g^{-1}	***	***	ns
Malic acid	mg g^{-1}	***	**	ns
Citric acid	mg g^{-1}	***	ns	ns
Fructose	mg g^{-1}	ns	**	*
Glucose	mg g^{-1}	***	***	**
Sucrose	mg g^{-1}	***	**	***
Total phenols content	mg g^{-1} FW	***	***	ns
Antioxidant capacity	mg g^{-1} FW	***	**	***

The significance is designated by asterisks as follows: *, statistically significant differences at p-value below 0.05; **, statistically significant differences at p-value below 0.01; ***, statistically significant differences at p-value below 0.001; ns, not significant.

Table 4. Mean values (2015 and 2016 seasons) for fruit quality and biochemical traits of ‘Big Top’ nectarine grafted on twenty rootstocks.

Experimental factor	FW	SSC	TA	DM	P	K	Ca	Mg	B	Fe	Zn	Mn	S	Ascorbic acid	Malic acid	Citric acid	Fructose	Glucose	Sucrose	TPC	Antioxidant capacity
<u>*Year*</u>																					
2015	160.7	13.7	5.2	15.1	23.6	219.4	5.5	7.9	2.4	11.6	10.9	2.9	0.06	2.9	2.9	1.4	8.6	12.5	69.8	1.2	0.36
2016	152.0	12.9	4.6	16.9	26.9	244.7	6.2	10.9	2.4	11.9	9.9	3.4	0.09	3.7	3.8	2.8	7.9	9.2	86.9	0.9	0.46
LSD P <0.05	*6.5*	*0.7*	*0.4*	*1.0*	*1.6*	*11.6*	*0.4*	*0.5*	*0.3*	*2.2*	*1.6*	*0.4*	*0.02*	*0.4*	*0.2*	*0.3*	*1.1*	*1.0*	*8.9*	*0.1*	*0.2*
<u>*Rootstock*</u>																					
AD-105	156.2	12.9	5,4	15.8	27.4	262.6	6.6	10.2	1.8	12.1	16.6	3.8	0.10	3.5	3.4	1.8	8.1	10.1	64.9	0.4	1.0

Adesoto® 101	158.6	12.9	4,5	15.7	25.5	240.0	5.0	9.5	1.8	9.5	10.0	3.5	0.10	3.1	3.1	1.7	8.7	10.2	75.9	0.4	1.1
Cadaman® Avimag	157.7	11.9	4,9	13.8	22.6	213.2	5.0	8.7	2.6	10.7	9.0	2.9	0.06	2.6	2.9	1.8	9.9	8.9	63.8	0.3	0.8
Castore	161.1	11.9	4,8	15.5	26.5	215.8	6.5	9.1	1.9	14.2	11.1	3.4	0.10	2.7	3.3	2.3	7.8	11.6	75.8	0.4	1.2
Controller-5	137.1	14.6	3,8	17.8	24.4	227.2	6.4	9.6	1.9	10.0	8.1	2.2	0.05	3.9	2.8	1.7	9.8	10.9	83.5	0.4	1.0
Controller-9	146.7	13.3	3,9	15.1	22	216.5	5.4	7.9	2.7	8.8	8.2	2.1	0.04	2.8	2.6	1.7	7.9	11.4	78.0	0.3	1.4
GF-677	156.7	12.6	4,8	15.3	25.9	219.1	5.3	9.2	2.2	10.1	10.0	2.9	0.09	3.3	3.3	1.9	8.1	10.3	76.7	0.4	0.9
IRTA-1	154.8	14.6	5,3	15.2	23.7	235.2	6.7	9.8	2.8	9.2	10.5	3.4	0.10	3.3	3.7	1.9	9.2	12.1	103.2	0.3	1.0
Isthara	158.3	12.1	4,9	17.9	21.0	240.3	6.0	8.7	2.7	11.1	9.7	2.7	0.04	3.1	3.4	3.3	7.4	10.5	73.7	0.4	1.3
Krymsk-1	145.5	16.4	4,6	18.0	28.1	228.2	5.6	9.9	1.9	12.1	9.9	2.6	0.06	4.1	3.4	1.9	10.3	12.8	94.4	0.6	1.6
Pacer-01.36	151.7	14.5	4,7	15.5	22.1	224.4	5.9	9.7	2.8	9.1	10.7	3.2	0.07	2.6	3.2	2.8	7.8	11.7	79.2	0.4	1.1
Padac-04.03	157.5	12.7	4,7	15.3	23.6	211.4	5.3	8.7	2.8	12.7	10.4	3.2	0.10	3.2	3.3	2.7	9.6	11.4	80.6	0.4	1.1
Padac-150	168.6	14.1	5,4	17.5	27.7	241.3	5.3	9.4	1.6	10.7	10.5	3.5	0.10	3.6	3.6	1.3	8.6	12.0	70.2	0.5	1.1
Penta	164.8	12.6	5,1	15.1	25.5	241.4	5.9	8.9	2.5	13.6	11.2	4.1	0.10	3.1	3.3	1.7	5.9	11.4	80.1	0.4	0.9
Polluce	158.6	12.9	4,8	14.6	26.6	224.4	5.9	9.1	2.2	14.4	9.4	2.9	0.10	3.5	3.7	2.2	6.8	8.8	81.1	0.3	1.1
PS	144.9	14.3	5,2	18.8	28.9	256.4	6.7	10.5	2.8	10.2	10.5	3.0	0.09	5.0	4.2	1.8	10.8	12.5	76.5	0.5	1.5
Rootpac® 20	151.5	13.7	4,8	18	26.4	238.0	5.4	9.3	2.5	8.4	9.5	3.0	0.10	3.6	3.5	1.8	7.9	12.5	83.8	0.5	1.0
Rootpac® 40	170.1	11.1	4,8	13.3	25.3	221.8	6.4	9.6	2.7	15.8	13.0	4.4	0.10	2.6	3.5	2.2	7.1	8.8	83.1	0.3	1.0
Rootpac® 70	165.0	12.1	5,4	14.5	23.9	228.3	6.1	9.7	3.4	14.4	10.0	2.8	0.08	2.7	3.4	2.2	6.3	9.9	64.1	0.4	1.0
Tetra	163.4	13.0	5,7	16.5	28.4	258.4	5.7	9.6	2.3	16.5	10.8	3.7	0.08	3.2	3.8	2.1	6.5	10.5	79.9	0.3	0.9
LSD P <0.05	*16.9*	*1.4*	*1.2*	*1.7*	*3.5*	*28.4*	*1.0*	*1.9*	*0.3*	*3.5*	*2.8*	*0.7*	*0.03*	*1.0*	*0.7*	*1.1*	*2.4*	*2.7*	*20.4*	*0.1*	*0.4*

Abbreviations: DM, dry matter; FW, fruit weight; SSC, soluble solids content; TA, titratable acidity; TPC; total phenolics content.

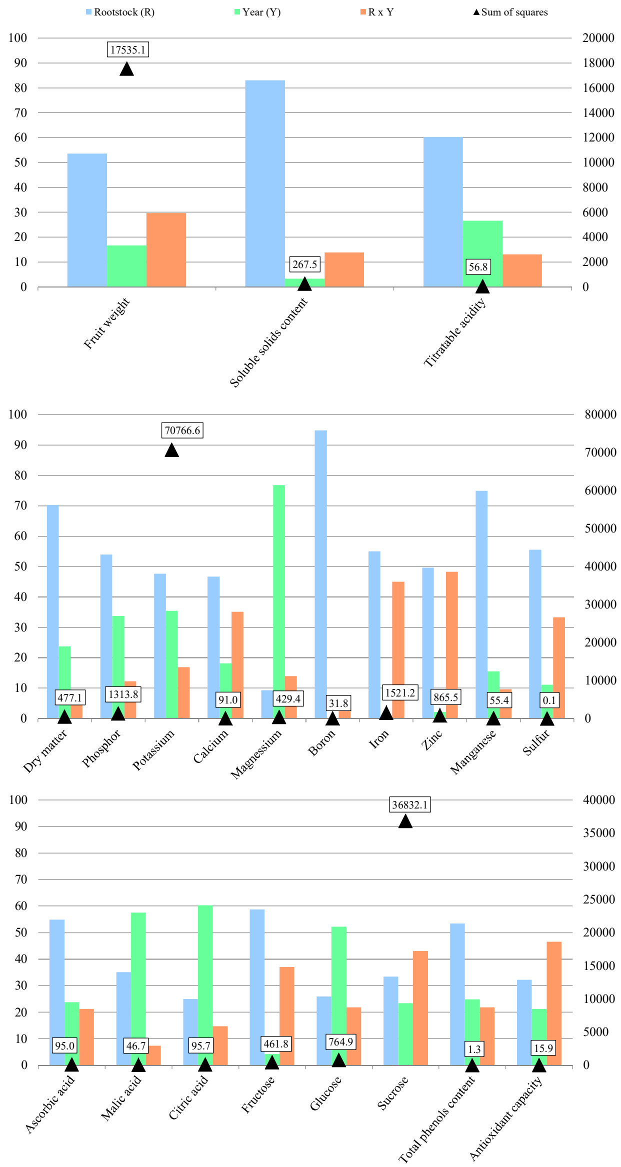

Figure 1. Reported variability for the traits evaluated on ‘Big Top’ grafted on 20 rootstocks. Triangles (Δ) show the total variability of each parameter by using the total sum of squares (right axis) after two-way ANOVA. Color bars for each trait show the variability expressed as percentage (%) of the total sum of squares for rootstock (R), year (Y) and the interaction of both (R ×Y) (left axis).

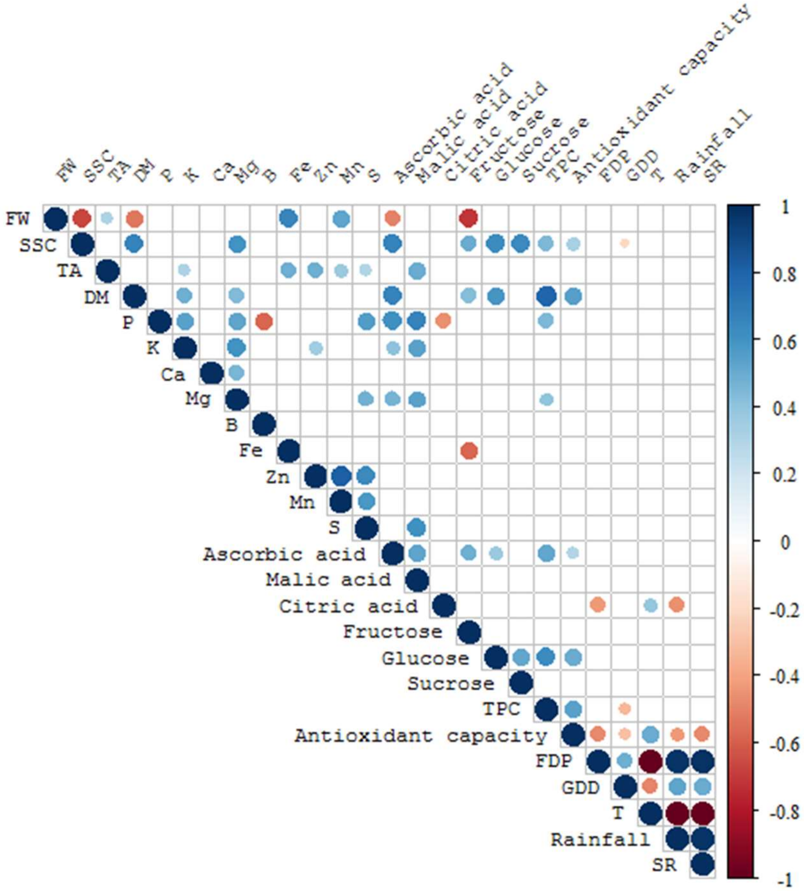

Figure 2. Bivariate correlations among the different quality and biochemical traits of ‘Big Top’ nectarine grafted on 20 rootstocks. *Abbreviations:* DM, dry matter; FDP, fruit development period; FF, flesh firmness; FW, fruit weight; GDD, growing degree days; SSC, soluble solids content; SR, accumulate solar radiation; T, mean daily temperature; TA, titratable acidity; TPC, total phenolics content. The size of the circle for each correlation and the color depict the significance and the correlation coefficient, respectively.

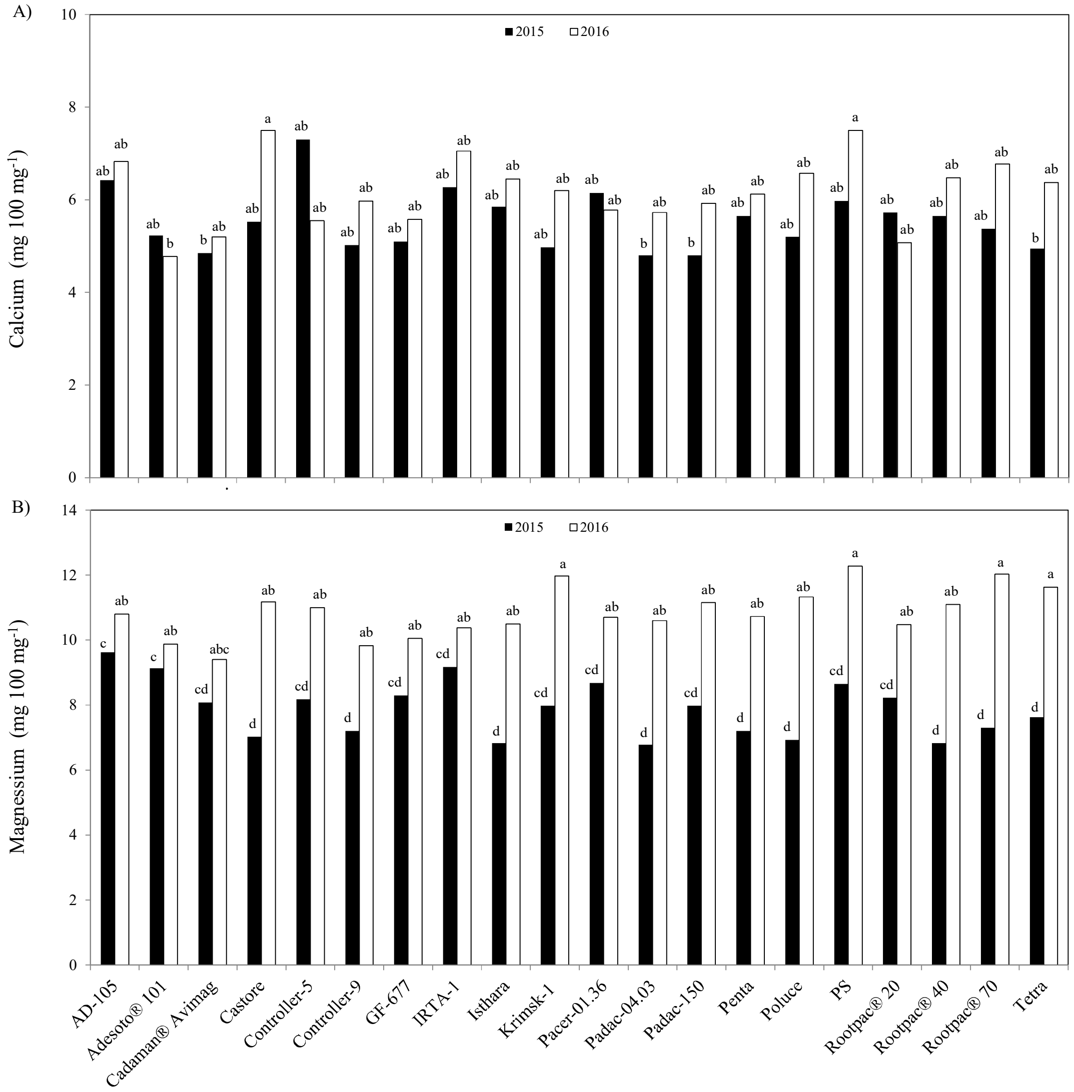

Figure 3. Fruit calcium and magnesium content of ‘Big Top’ nectarine, grafted on twenty rootstocks in 2015 and 2016. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Tukey HSD Test.

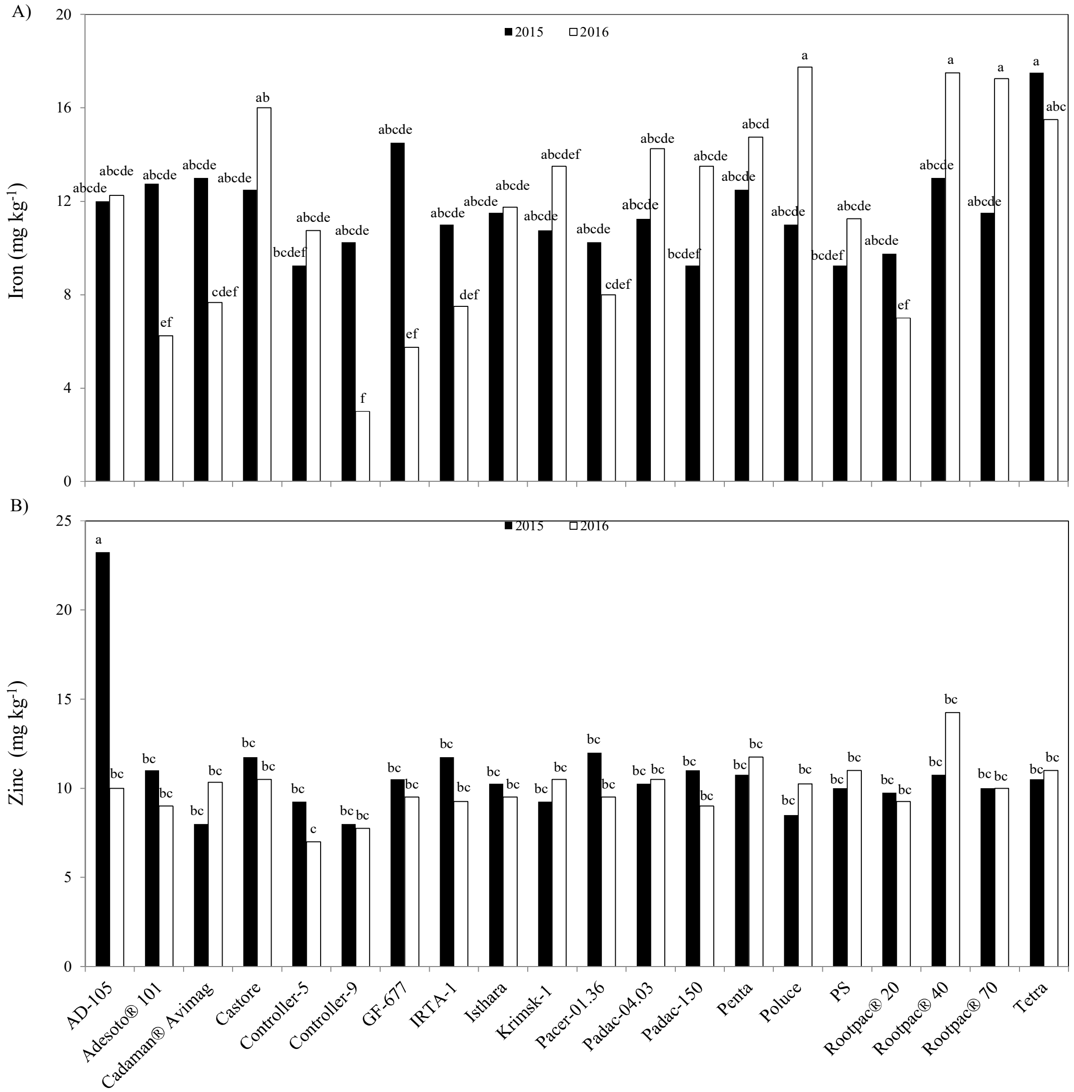

Figure 4. Fruit iron and zinc content of ‘Big Top’ nectarine grafted on twenty *Prunus* rootstocks in 2015 and 2016. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

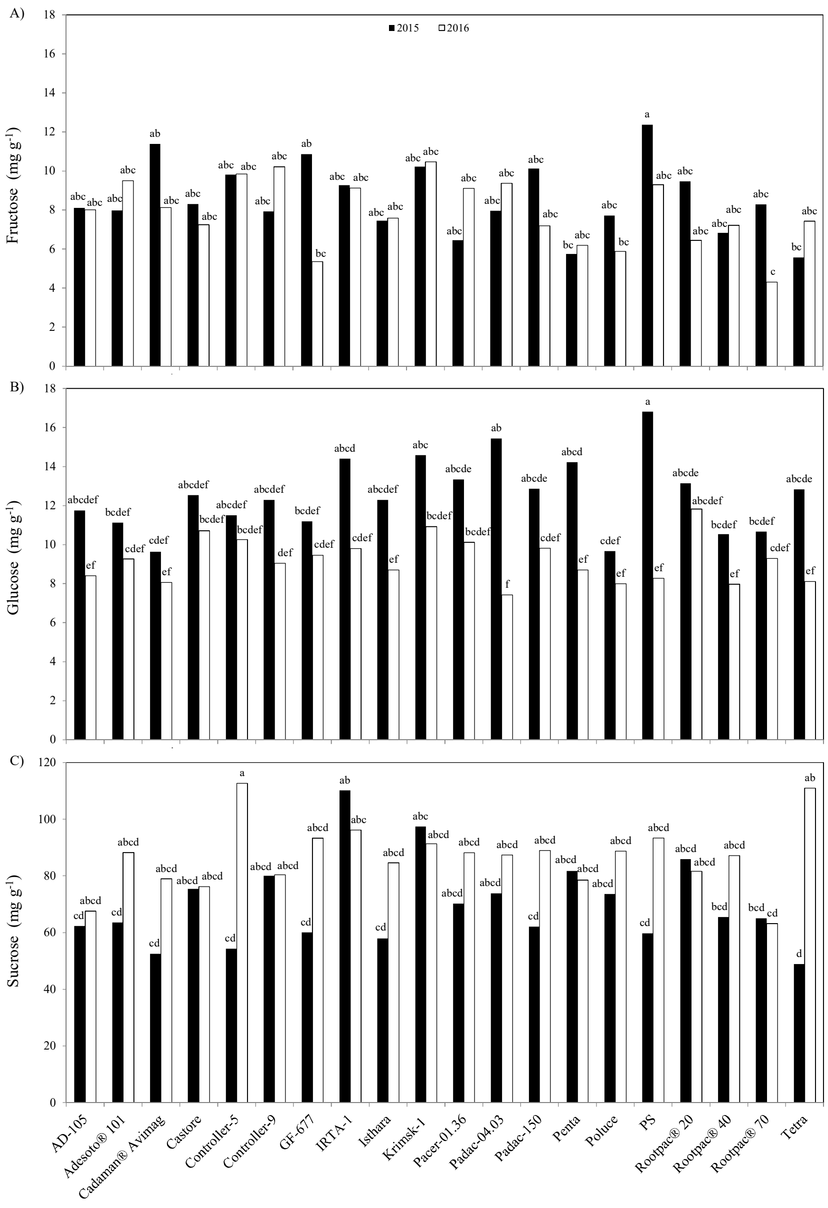

Figure 5. Individual sugars (fructose, glucose and sucrose) content of ‘Big Top’ fruit grafted on twenty rootstocks in 2015 and 2016. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Tukey HSD Test.

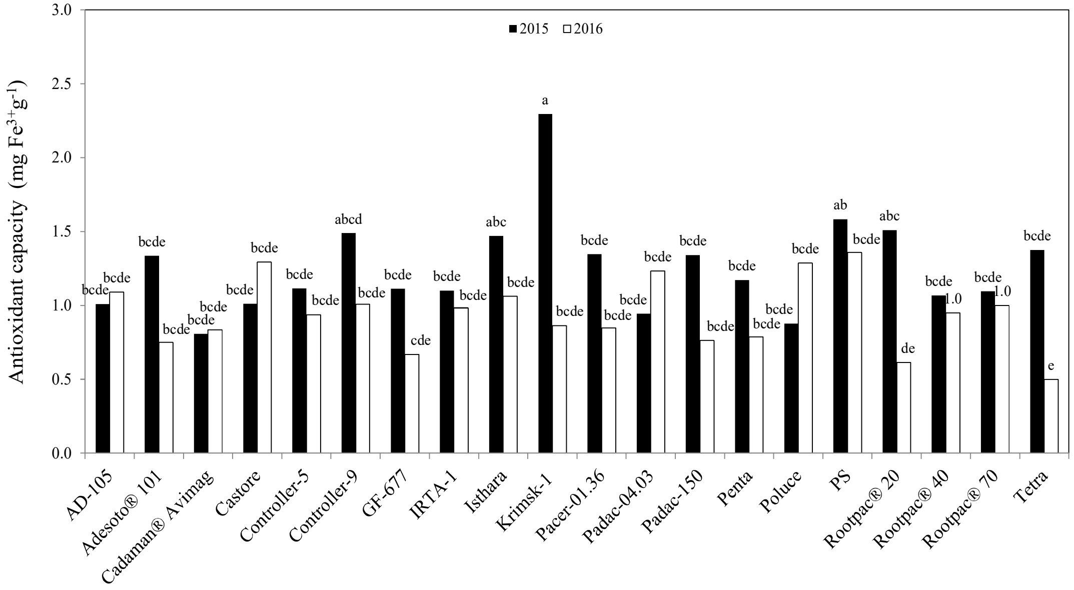

Figure 6. Antioxidant capacity of ‘Big Top’ fruit grafted on twenty rootstocks in 2015 and 2016. Means followed by the same letter in each column are not significantly different at *P* ≤ 0.05 according to Tukey HSD Test.