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Effects of Rootstock/Scion Combinations on the Flavor of Citrus Fruit

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ABSTRACT: We examined sensory quality, total soluble solids (TSS) and acidity levels, and aroma volatiles compositions of 'Or' and 'Odem' mandarins grafted on sour orange (SO), Volkamer lemon (Volka), and US-812 rootstocks; 'Valencia' oranges grafted on SO, Volka, and ×639 rootstocks; and a new pummelo × grapefruit hybrid cv. 'Redson' grafted on SO, Volka, and macrophylla rootstocks. TSS and acidity levels of all species were lower in juice of fruits on Volka than on SO. Sensory quality evaluations revealed that 'Odem' mandarins and 'Redson' fruits grown on SO were preferred to those on Volka but the rootstocks had no notable effects on flavor perception of 'Or' mandarins and 'Valencia' oranges. Chromatographic analysis revealed that contents of aroma volatiles, especially terpenes, in homogenized segments of 'Odem' and 'Redson' but not of 'Or' and 'Valencia' were significantly lower on Volka than on SO. Overall, the effects of rootstocks on citrus fruit flavor depended on specific rootstock/scion interactions. Furthermore, the flavor of some varieties grown on Volka was inferior to that on SO because of lower TSS and acidity levels and lower aroma volatiles contents.

KEYWORDS: *aroma, citrus, flavor, mandarin, rootstock, sour orange, Volka*

INTRODUCTION

Selection of rootstocks is crucial for commercial cultivation of citrus fruit, as they affect productivity (yield and fruit size); resistance to pests (nematodes and weevils), diseases (phytophthora rots, tristeza virus, exocortis and xyloporosis viroids), and environmental stresses (drought, frost, salinity, flooding); compatibility with soils (e.g., clay soil, and high-pH soil); growth rates; and so forth.[1−3] In addition, rootstocks may affect internal quality parameters of citrus fruit, including juice total soluble solids (TSS) and acidity levels, as well as vitamin C content and antioxidant capacity.[4−7] For example, TSS levels of 'Valencia' oranges (*Citrus sinensis* L. Osbeck) on Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*) were greater than 20% higher than those grafted on rough lemon.[4]

Nevertheless, despite the great importance of fruit flavor for commercial marketing of citrus fruits, relatively little is yet known regarding the possible effects of rootstock/scion combinations on the flavor quality of citrus fruits or about their effects on aroma volatiles contents and composition. To the best of our knowledge, the only professional sensory analysis test conducted so far in order to evaluate the effects of rootstocks on citrus fruit sensory quality was performed with 'Valencia' oranges, and taste panelists were not able to distinguish among the flavors of pasteurized juices produced from trees on Carrizo citrange, Cleopatra mandarin (*C. reticulate* Blanco), rough lemon (*C. jhambiri* Lush.), sour orange (*C. aurantium* L.) (SO), and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) rootstocks that were normalized against TSS/acid ratios.[2] As far as we know, little information is available regarding possible effects of rootstock/scion combinations on production of aroma volatiles in citrus. In bergamot fruits, it was found that rootstocks could influence the aroma volatiles composition of peel essential oils; it was noted that fruit on ×639 and Volkamer lemon (*C. volkameriana* Ten. and Pasq.) (Volka) rootstocks had similar proportions of oxygenated compounds (mainly linalool and linalyl acetate) and hydrocarbons (mainly limonene) to those of fruits on SO rootstock.[8] More recently, it was reported that essential oils from juice and peel of 'Page' tangelos on Swingle citrumelo and Yuzu (*C. ichangensis* swing. × *C. reticulata*) rootstocks had slightly higher aldehyde levels than those on other tested rootstocks, such as SO and Troyer citrange (*C. sinensis* × *P. trifoliata*).[9] Nevertheless, rootstocks had only minor effects on aroma volatiles compositions of 'Page' tangelo flower and leaf tissues.[10]

Until now, the rootstock most commonly used for cultivation of citrus fruits in Israel has been SO, which is known for providing high-quality, tasty fruit but is sensitive to tristeza virus disease and therefore is being replaced worldwide with other, more tolerant rootstocks.[11] Other commercially important rootstocks used in Israel are Volka and *Citrus macrophylla* (macrophylla), which provide high yields and compatibility to calcareous soils but are considered to provide somewhat lower-quality fruit, at least in terms of measurable TSS and acidity levels.[1,12,13] Other important new rootstocks in Israel are US-812 (*C. reticulata* × *P. trifoliata*) and ×639 (*C. reticulata* × *P. trifoliata*), which provide high yields and high-quality fruits.[13,14]

The perceived flavor of citrus fruits results from combinations of taste and aroma sensations, in which the sweet and sour taste attributes are principally governed by the presence of sugars and acids in the juice sacs, and the aroma of the fruit evolves from a mixture of dozens of volatiles that provide various fruity, floral, terpene, citrus, green/grassy, fatty, metallic, herbal, and other notes.[15,16] In the present study, we evaluated the effects of various rootstocks on sensory quality, TSS and

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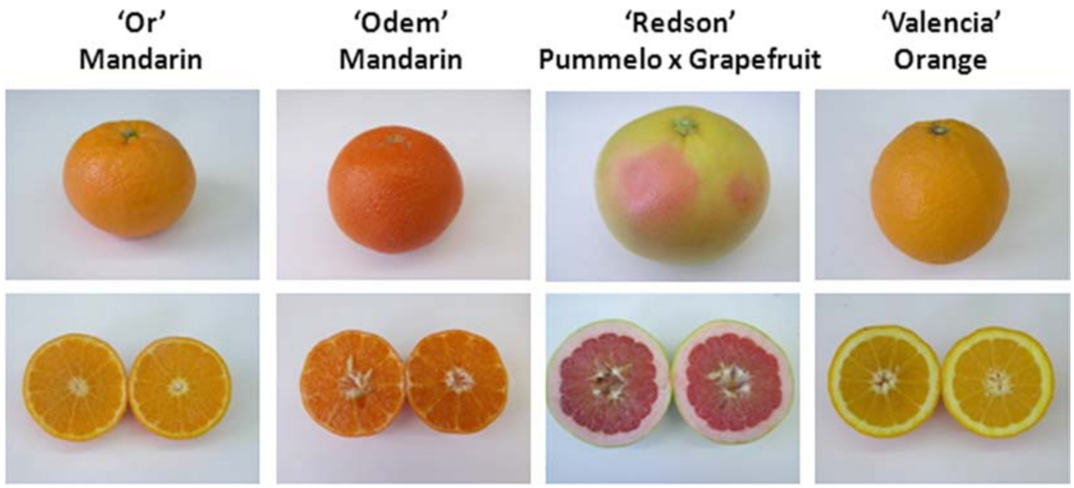

Figure 1. Photographs of 'Or' and 'Odem' mandarins, a new pummelo × grapefruit hybrid cv. 'Redson', and a 'Valencia' orange.

acidity levels, and aroma volatiles contents and compositions of 'Or' and 'Odem' mandarins (*C. reticulata* Blanco), 'Valencia' oranges, and a new pummelo (*C. maxima* Burm.) × grapefruit (*C. paradisi* Macf.) hybrid, cv. 'Redson'.

■ MATERIALS AND METHODS

Plant Material. The experiments were conducted on four citrus varieties: 'Or' and 'Odem' mandarins (*C. reticulata* Blanco), 'Valencia' oranges (*C. sinensis* L. Osbeck), and a new pummelo (*C. maxima* (Burm.) Merr., cv. 'Chandler') × grapefruit (*C. paradisi* Macf., cv. 'Hudson') hybrid, cv. 'Redson' (Figure 1). The rootstock/scion combinations tested were as follows: 'Or' and 'Odem' mandarins grafted on sour orange (*C. aurantium* L.) (designated SO), Volkamer lemon (*C. volkameriana* Ten. and Pasq.) (designated Volka), US-812 (a hybrid between 'Sunki' mandarin (*C. reticulata* Blanco) and 'Benecke' trifoliate orange (*P. trifoliata* L. ref.)) rootstocks; 'Valencia' oranges grafted on SO, Volka, and ×639 (a hybrid between 'Cleopatra' mandarin (*C. reticulata* Blanco) and 'Benecke' trifoliate orange (*P. trifoliata* L. ref.)) rootstocks; and the new pummelo × grapefruit hybrid cv. 'Redson' grafted on SO, Volka, and *C. macrophylla* (*C. macrophylla* Wester) rootstocks. The fruits were harvested from experimental rootstock/scion evaluation trials conducted under standard commercial orchard management procedures. The fruits were harvested at optimal maturity, at the peak of the commercial harvest season of each variety. All experiments were repeated twice during the 2011/2012 and 2012/2013 citrus growing seasons. The provided data are of one out of two representing experiments with similar results, in which 'Or' mandarins were harvested on Feb. 10, 2013, 'Odem' mandarins were harvested on Jan. 3, 2012, the pummelo × grapefruit hybrid cv. 'Redson' fruit were harvested on Dec. 17, 2012, and 'Valencia' oranges were harvested on April 19, 2012. The 'Or' mandarins were harvested from 6 year old trees, 'Odem' mandarins from 7 year old trees, 'Redson' fruit from 6 year old trees, and 'Valencia' oranges from 13 year old trees.

Chemicals. Chemical standards of the volatiles acetaldehyde, ethyl acetate, ethyl propanoate, ethyl octanoate, ethyl decanoate, hexanal, hexanol, *cis*-3-hexenol, nonanal, 4-terpineol, and *α*-terpineol were purchased from the Sigma Flavor & Fragrances Catalogue (Sigma−Aldrich, St Louis, MO, USA). Ethanol (GC grade, 99.8%) was purchased from the Sigma Chemical Catalog (Sigma−Aldrich).

Juice TSS and Acid Contents. TSS content in the juice was determined with a PAL-1 digital refractometer (Atago, Tokyo, Japan) and acid content was assessed by titration to pH 8.3 with 0.1 N NaOH by means of a CH-9101 automatic titrator (Metrohm Herisau, Switzerland). TSS and acidity measurements included five replications, each of juice collected from three different fruits.

Analysis of Aroma Volatiles. Aroma volatiles were extracted from homogenized citrus fruit segments as described previously.[17−19]

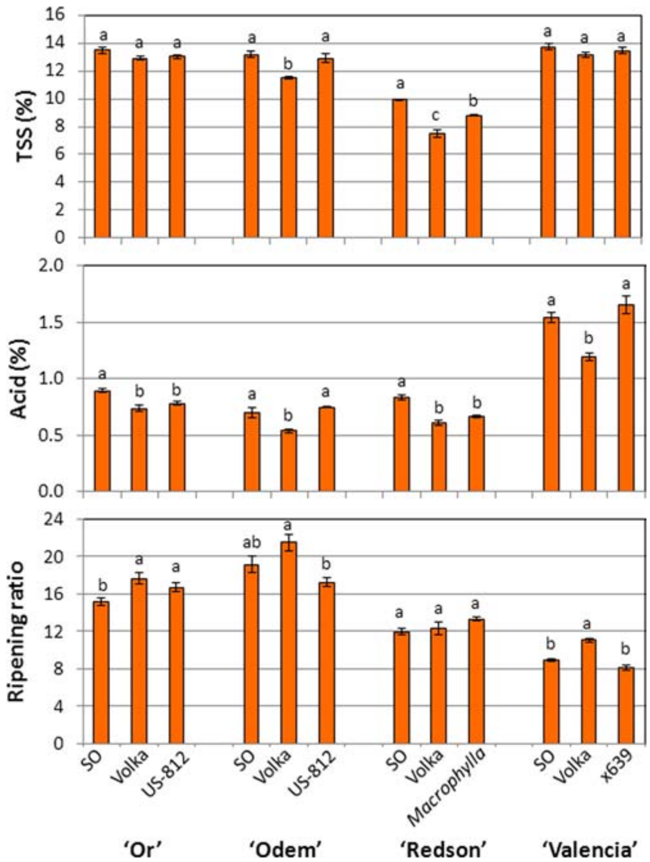

Figure 2. Effects of various rootstocks on TSS and acid contents and ripening ratios of 'Or' and 'Odem' mandarins, a new pummelo × grapefruit hybrid cv. 'Redson', and 'Valencia' oranges. Data are means ± SE of five measurements, each of juice from three different fruits. Different letters indicate significant differences among rootstocks within each variety at $P \leq 0.05$.

The fruits were hand peeled, weighed, and blended for 30 s with an equal weight of 30% NaCl to inhibit enzymatic degradation. Aliquots (2 mL) were placed in 10 mL glass vials, and 5 μL of 1-pentanol (Sigma−Aldrich, St Louis, MO, USA) diluted 1:1000 (v/v) in water was added as an internal standard. The vials were stored at −20 °C pending analysis. In all cases, aroma volatiles were determined in three replicate measurements, each involving three different fruits, that is, a total of nine fruits per treatment.

Table 1. Effects of Various Rootstocks on Aroma Volatiles Contents of 'Or' Mandarins[a]

compd	RI[b]	RI[c]	conc (μg L^{-1}) SO	Volka	US-812	odor description[d]
Alcohols						
ethanol	668	668	25 a	23 a	28 a	sweet
3-hexen-1-ol	851	855	15 a	24 a	21 a	fresh, green, grassy
1-octanol	1069	1072	18 a	12 a	17 a	chemical, metal, burnt
linalool	1098	1100	8 a	6 a	7 a	flower, lavender
4-terpineol	1177	1179	1 a	0 a	2 a	turpentine, must
α-terpineol	1190	1195	2 a	1 a	2 a	oil, anise, mint
Aldehydes						
pentanal	694	706	94 a	98 a	82 a	almond, malt, pungent
hexanal	798	801	577 a	568 a	539 a	grass, tallow, fat
(*E*)-2-hexenal	848	844	52 a	76 a	99 a	green, leaf
heptanal	900	903	19 a	9 a	7 a	fat, citrus, rancid
octanal	1002	1006	42 a	29 a	26 a	fat, soap, lemon, green
nonanal	1103	1104	**34 a**	**11 b**	**0 c**	fat, citrus, green
decanal	1204	1209	**17 a**	**8 b**	**9 b**	orange peel, tallow
perillaldehyde	1275	1271	153 a	114 a	217 a	spice
Esters						
ethyl acetate	608	600	59 a	67 a	78 a	pineapple
ethyl propanoate	707	713	10 a	11 a	11 a	fruit
ethyl hexanoate	998	1002	50 a	40 a	52 a	apple peel, fruit
ethyl octanoate	1196	1198	7 a	4 a	5 a	fruit, fat
Ketones						
carvone	1244	1254	2 a	1 a	1 a	mint
Monoterpenes						
α-pinene	931	939	353 a	222 a	292 a	pine, turpentine
sabinene	972	972	33 a	21 a	27 a	turpentine, wood
β-pinene	990	981	2269 a	1,368 a	2463 a	pine, resin, turpentine
α- fellandrene	1007	1007	1356 a	856 a	1130 a	turpentine, mint, spice
limonene	1034	1033	427 555 a	305 671 a	447 784 a	lemon, orange
(*Z*)-β-ocimene	1047	1043	514 a	284 a	505 a	citrus, herb, flower
γ-terpinene	1058	1060	743 a	402 a	642 a	gasoline, turpentine
δ-terpinene	1088	1070	2326 a	1659 a	2488 a	pine, plastic
Sesquiterpenes						
α-cubebene	1351	1345	251 a	142 a	212 a	herb, wax
copaene	1378	1377	266 a	141 a	250 a	wood, spice
valencene	1496	1490	176 a	121 a	171 a	green, oil
δ-cadinene	1529	1519	483 a	273 a	318 a	thyme, wood
total volatiles			437 511 a	312 265 a	456 738 a	

[a]Volatiles whose concentrations were significantly different ($P \leq 0.05$) among rootstocks are in bold. [b]Calculated retention indices based on a series of *n*-alkanes. [c]Published retention indices on DB-5 column according to the Flavornet database (www.flavornet.org). [d]Odor descriptions according to the Flavornet (http://www.flavornet.org and the University of Florida Citrus Flavor Database (http://www.crec.ifas.ufl.edu/rouseff).

Aroma volatiles were identified by gas chromatography (GC) coupled with mass spectrometry (MS). Prior to analysis, the samples were thawed at room temperature and were allowed to equilibrate for 5 min at 40 °C. Afterward, volatiles were extracted from the vial headspaces by solid-phase microextraction (SPME) using 1 cm long stable flexible fibers coated with a 50/30 μm layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA, USA). Volatiles were extracted from the vial headspaces during incubation at the same temperature (40 °C) for an additional 25 min. The extracted volatiles were injected into a model 7890A gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with an HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA), by desorption for 2 min at 250 °C into the splitless inlet, by means of an autosampler (CTC PAL, Zwingen, Switzerland). The oven was programmed to run at 50 °C for 1 min, then to ramp up to 160 °C at 5 °C min^{-1}, then up to 260 °C at 20 °C min^{-1}, and finally to remain at that temperature for 4 min. The helium carrier gas flow was set at 0.8 mL/min. The effluent was transferred to a model 5975C mass spectrometer detector (Agilent) that was set to scan the m/z range from 40 to 206 at 7.72 scans s^{-1} in positive ion mode, and mass spectra in electron impact mode were generated at 70 eV. Chromatograph peaks were identified by comparing the mass spectrum of each component with the U.S. National Institute of Standards and Technology (NIST) 2006 Mass Spectral Library. Identification of aroma volatiles was further confirmed by calculating their linear retention indices (RI) by using a series of *n*-alkanes (C5−C20) and comparing their values with various published databases, mainly that of Flavornet (www.flavornet.org) and the University of Florida Citrus Flavor Database (http://www.crec.ifas.ufl.edu/rouseff/). The identities of some of the volatiles—acetaldehyde, ethanol, ethyl acetate, ethyl propanoate, ethyl octanoate, ethyl decanoate, hexanal, hexanol, *cis*-3-hexenol, nonanal, 4-terpineol, and α-terpineol—were further confirmed by comparison with authentic chemical standards (Sigma−Aldrich).

The detected volatiles were semiquantified by comparison of their observed peak areas with those of chemical standards: ethanol and 1-pentanol were used for quantifying alcohols, heptanal for aldehydes, ethyl acetate for esters, limonene for monoterpenes, linalool for terpene alcohols, valencene for sequiterpenes, and carvone for ketones.

Table 2. Effects of Various Rootstocks on Aroma Volatiles Contents of 'Odem' Mandarins[a]

compd	RI[b]	RI[c]	conc (μg L^{-1}) SO	Volka	US-812	odor description[d]
Alcohols						
ethanol	668	668	6 a	10 a	34 a	sweet
1-octanol	1070	1072	10 a	11 a	42 a	chemical, metal, burnt
linalool	1100	1100	**129 ab**	**67 b**	**205 a**	flower, lavender
4-terpineol	1179	1179	6 a	4 a	14 a	turpentine, nutmeg
α-terpineol	1192	1195	8 a	4 a	12 a	oil, anise, mint
Aldehydes						
pentanal	697	706	9 a	12 a	22 a	almond, malt, pungent
hexanal	800	801	132 a	159 a	227 a	grass, tallow, fat
heptanal	901	903	12 a	13 a	29 a	fat, citrus, rancid
(*Z*)-2-heptenal	955	951	2a	5 a	8 a	green
octanal	1003	1006	18 a	18	40	fat, soap, lemon, green
(*E*)-2-octenal	1057	1060	3 a	9 a	9 a	green
nonanal	1104	1104	9 a	13 a	19 a	fat, citrus, green
(*E*)-2-nonenal	1160	1154	0 a	3 a	2 a	paper
decanal	1206	1209	11 a	8 a	29 a	soap, orange peel
perillaldehyde	1277	1271	**4 ab**	**0 b**	**14 a**	spice
dodecanal	1410	1407	**7 ab**	**0 b**	**14 a**	sweet, waxy
Esters						
ethyl propanoate	709	713	45 a	0 a	85 a	fruit
Ketones						
1-penten-3-one	684	680	0 a	4 a	0 a	fish, pungent
dihydrocarvone	1199	1200	0 a	0 a	4 a	herb, warm
carvone	1247	1254	5 a	6 a	11 a	mint
Monoterpenes						
α-pinene	933	939	500 a	0 a	1079 a	pine, turpentine
β-pinene	991	981	**1571 ab**	**578 b**	**3872 a**	pine, resin, turpentine
1,3,8-*p*-menthatriene	1025	1115	887 a	617 a	2170 a	turpentine
limonene	1030	1033	**144 251 ab**	**80 134 b**	**220 130 a**	lemon, orange
(*Z*)-β-ocimene	1048	1043	858 a	0 a	611 a	citrus, herb, flower
γ-terpinene	1059	1074	**738 ab**	**0 b**	**901 a**	gasoline, turpentine
total volatiles			**149 225 ab**	**81 673 b**	**229 603 a**	

[a]Volatiles whose concentrations were significantly different ($P \leq 0.05$) among rootstocks are in bold. [b]Calculated retention indices based on a series of *n*-alkanes. [c]Published retention indices on DB-5 column according to the Flavornet database (www.flavornet.org). [d]Odor descriptions according to the Flavornet (http://www.flavornet.org and the University of Florida Citrus Flavor Database (http://www.crec.ifas.ufl.edu/rouseff).

Sensory Evaluation. Sensory quality was evaluated by means of various preference, descriptive, and discrimination tests.[20] In all cases, fruits were hand peeled, and separated segments were cut into halves and placed in glass cups identified by randomly assigned three-digit codes; each treatment comprised a mixture of six to eight cut segments prepared from six different fruits, per panelist. Flavor preference was evaluated according to a nine-point hedonic scale from "very strong dislike" to "very strong like", and this acceptance test was conducted by 35−40 staff members and students working in the Department of Postharvest Science at the ARO, the Volcani Center. Quantitative descriptive flavor analyses were performed by a trained sensory panel comprising 10 members, five males and five females aged 25−62, who routinely perform taste tests of citrus fruits. Each panelist assessed the various attributes of the samples according to an unstructured 100 mm linear scale for each attribute. The scale ranged from "very weak" to "very strong", and sensory data were recorded as distances (mm) from the origin. The chosen sensory attributes were sweet, sour, bitter, fruity, juicy, and gummy. Discrimination tests (triangle tests) of fruits grafted on SO against those on Volka were conducted among 35−45 untrained panelists; half of the tests included two samples of fruit on SO and one sample on Volka, and the second half included two samples on Volka and one on SO.

Statistical Analysis. One-way ANOVA and Tukey's HSD pairwise comparison tests were applied by means of the JMP statistical software, version 7 (SAS Institute Inc., Cary, NC, USA) and the Microsoft Office Excel program. The significance of the triangle test results was determined by chi-square analysis, and that of the flavor preference results by binomial distribution tests.

■ RESULTS

Effects of Rootstock/Scion Combinations on Juice TSS and Acid Contents. In all citrus fruits tested, including 'Or' and 'Odem' mandarins, the pummelo × grapefruit hybrid 'Redson', and 'Valencia' oranges (Figure 1), it was found that TSS and acidity levels in juice of fruits grown on Volka rootstock were lower than those in fruits on SO, US-812, and ×639 rootstocks (Figure 2). For example, TSS levels in juice of various fruits on SO and Volka were as follows: in 'Or' mandarins, 13.5 and 12.9%, respectively; in 'Odem' mandarins, 13.2 and 11.5%, respectively; in 'Redson', 10.0 and 7.5%, respectively; and in 'Valencia' oranges, 13.8 and 13.2%, respectively (Figure 2). Also, acidity levels in juice of the same fruits on SO and Volka were as follows: in 'Or' mandarins, 0.9 and 0.7%, respectively; in 'Odem' mandarins, 0.7 and 0.5%, respectively; in 'Redson', 0.8 and 0.6%, respectively; and in 'Valencia' oranges, 1.5 and 1.2%, respectively (Figure 2). Since fruits grown on Volka rootstock had lower levels of both TSS and acidity than those on SO, then overall TSS/acidity ratios, that is, "ripening ratios", hardly differed from or were just

Table 3. Effects of Various Rootstocks on Aroma Volatiles Contents of the Pummelo × Grapefruit Hybrid cv. 'Redson'[a]

compd	RI[b]	RI[c]	conc (μg L^{-1}) SO	Volka	macrophylla	odor description[d]
Alcohols						
1-octanol	1069	1072	49 a	20 a	41 a	chemical, metal, burnt
linalool	1098	1100	**21 a**	**9 b**	**7 b**	flower, lavender
4-terpineol	1177	1179	4 a	2 a	1 a	turpentine, nutmeg, must
α-terpineol	1190	1195	**11 a**	**5 b**	**3 b**	oil, anise, mint
Aldehydes						
hexanal	798	801	**27 a**	**15 b**	**18 b**	grass, tallow, fat
(*E*)-2-hexenal	848	844	**6 a**	**0 b**	**0 b**	green, leaf
heptanal	900	903	5 a	5 a	7 a	fat, citrus, rancid
octanal	1002	1006	51 a	14 a	13 a	fat, soap, lemon, green
nonanal	1103	1104	6 a	2 a	2 a	fat, citrus, green
decanal	1204	1209	**23 a**	**9 b**	**7 b**	soap, orange peel
neral	1240	1247	**11 a**	**3 b**	**2 b**	lemon
geranial	1270	1277	**18 a**	**6 b**	**3 b**	lemon, mint
Ketones						
carvone	1244	1254	**5 a**	**2 b**	**1 b**	mint
Monoterpenes						
α-pinene	932	939	3595 a	1782 a	1822 a	pine, turpentine
sabinene	972	972	**1065 a**	**0 b**	**0 b**	turpentine, wood
β-pinene	990	981	**36 263 a**	**14 465 b**	**16 442 b**	pine, resin, turpentine
limonene	1034	1033	**623 267 a**	**350 284 b**	**411 858 b**	lemon, orange
cis-β-ocimene	1047	1043	1018 a	410 a	425 a	citrus, herb, flower
γ-terpinene	1058	1060	**1131 a**	**507 b**	**672 b**	gasoline, turpentine
δ-terpinene	1088	1088	5397 a	3457 a	4169 a	pine, plastic
Sesquiterpenes						
δ-elemene	1339	1340	7282 a	3307 a	1628 a	wood
α-cubebene	1351	1345	**3208 a**	**1231 b**	**719 b**	herb, wax
copaene	1378	1377	**7640 a**	**2318 b**	**1322 b**	wood, spice
β-elemene	1394	1393	**4472 a**	**1670 b**	**964 b**	herb, wax, fresh
β-caryophyllene	1458	1467	**12 234 a**	**5480 b**	**2753 b**	wood
γ-muurolene	1479	1475	**4428 a**	**1117 b**	**558 b**	herb, wood, spice
valencene	1497	1490	**5301 a**	**1467 b**	**1122 b**	green, oil
δ-cadinene	1530	1519	**12 375 a**	**4328 b**	**2733 b**	thyme, medicine, wood
total volatiles			**728 914 a**	**391 915 b**	**447 293 b**	

[a]Volatiles whose concentrations were significantly different ($P \leq 0.05$) among rootstocks are in bold. [b]Calculated retention indices based on a series of *n*-alkanes. [c]Published retention indices on DB-5 column according to the Flavornet database (www.flavornet.org). [d]Odor descriptions according to the Flavornet (http://www.flavornet.org and the University of Florida Citrus Flavor Database (http://www.crec.ifas.ufl.edu/rouseff).

slightly higher in fruits grown on Volka than in those on SO (Figure 2). In contrast to those of the fruits grown on SO, US-812, and ×639 rootstocks, the TSS and acidity levels of 'Redson' fruit on macrophylla rootstock were fairly similar to those of fruits on Volka (Figure 2).

Effects of Rootstock/Scion Combinations on Aroma Volatiles Contents. We examined the effects of rootstock/scion combinations on aroma volatiles contents and compositions in various citrus fruits. In 'Or' mandarins, we detected a total of 31 volatiles, comprising 6 alcohols, 8 aldehydes, 4 esters, 1 ketone, 8 monoterpenes, and 4 sesquiterpenes (Table 1). We noticed only minor effects of rootstocks on aroma volatiles composition: the levels of just two aldehydes, nonanal and decanal, were significantly higher in fruits on SO than in those on Volka and US-812 (Table 1). The total amount of aroma volatiles in 'Or' mandarins grafted on Volka was not significantly different from that in fruits on SO or US-812 rootstocks (Table 1).

In 'Odem' mandarins, we detected a total of 26 volatiles, comprising 5 alcohols, 11 aldehydes, 1 ester, 3 ketones, and 6 monoterpenes (Table 2), and we detected significant differences in the contents of 6 volatiles among fruits grown on different rootstocks. More specifically, we found that the levels of one terpene alcohol (linalool), two aldehydes (perillaldehyde and dodecanal), and three monoterpenes (β-pinene, limonene, and γ-terpinene) were significantly higher in fruits grown on US-812 rootstock than in those on Volka (Table 2). Overall, the total amount of aroma volatiles in 'Odem' mandarins grafted on Volka was significantly lower than that in those grown on US-812 rootstock (Table 2).

In the pummelo × grapefruit hybrid 'Redson', we detected a total of 28 volatiles, comprising 4 alcohols, 8 aldehydes, 1 ketone, 7 monoterpenes, and 8 sesquiterpenes (Table 3) and found significance differences in the contents of 19 volatiles between fruits grown on different rootstocks. More specifically, we found that the levels of two terpene alcohols (linalool and α-terpineol), five aldehydes (hexenal, (*E*)-2-hexenal, decanal, neral, and geranial), one ketone (carvone), four monoterpenes (sabinene, β-pinene, limonene, and γ-terpinene), and seven sesquiterpenes (α-cubebene, copaene, β-elemene, β-caryophyllene, γ-muurolene, valencene, and δ-cadinene) were significantly higher in fruits on SO rootstock than in those on Volka and

Table 4. Effects of Various Rootstocks on Aroma Volatiles Contents of 'Valencia' Oranges[a]

compd	RI[b]	RI[c]	conc (μg L^{-1}) SO	Volka	×639	odor description[d]
Alcohols						
ethanol	668	668	12 a	8 a	6 a	sweet
1-octanol	1070	1072	172 a	386 a	410 a	chemical, metal, burnt
linalool	1100	1100	41 a	68 a	83 a	flower, lavender
4-terpineol	1178	1179	10 a	15 a	23 a	turpentine, must
α-terpineol	1192	1195	8 a	10 a	14 a	oil, anise, mint
Aldehydes						
hexanal	799	801	181 a	137 a	90 a	grass, tallow, fat
heptanal	901	903	20 a	9 a	0 a	fat, citrus, rancid
octanal	1003	1006	376 a	585 a	670 a	fat, soap, lemon, green
nonanal	1104	1104	130 a	174 a	194 a	fat, soap, green
decanal	1205	1209	517 a	655 a	876 a	tallow
neral	1242	1247	3 a	6 a	6 a	lemon
geranial	1271	1277	3 a	5 a	7 a	lemon, mint
perillaldehyde	1276	1271	59 a	89 a	109 a	spice
dodecanal	1409	1407	5 a	10 a	11 a	sweet, waxy
Esters						
ethyl butanoate	800	794	85 a	39 a	33 a	fruity
ethyl hexanoate	999	1002	61 a	47 a	42 a	apple peel, fruit
ethyl octanoate	1197	1198	18 a	19 a	9 a	fruit, fat
octyl acetate	1211	1149	35 a	29 a	57 a	fruit
citronellyl acetate	1353	1357	5 a	5 a	9 a	rose, dust
Ketones						
carvone	1246	1254	**109 a**	**25 b**	**12 b**	mint
Monoterpenes						
α-pinene	933	939	**98 b**	**75 b**	**227 a**	pine, turpentine
sabinene	973	972	**21 b**	**33 b**	**103 a**	turpentine, wood
β-pinene	991	981	**1211 b**	**1499 b**	**2845 a**	pine, resin, turpentine
limonene	1037	1033	**317 700 b**	**309 379 b**	**613 502 a**	lemon, orange
(*Z*)-β-ocimene	1049	1043	842 a	316 a	517 a	citrus, herb, flower
γ-terpinene	1060	1074	565 a	782 a	1294 a	gasoline, turpentine
δ-terpinene	1089	1090	2037 a	2205 a	3548 a	pine, plastic
Sesquitterpenes						
copaene	1379	1377	**178 b**	**475 b**	**1268 a**	wood, spice
β-elemene	1395	1393	452 a	544 a	960 a	herb, wax, fresh
caryophyllene	1424	1432	433 a	776 a	1082 a	wood, spicy
valencene	1499	1490	19 200 a	10 748 a	18 877 a	green, oil
δ-cadinene	1531	1519	520 b	732 b	1963 a	thyme, wood
total volatiles			**343 840 b**	**328 419 b**	**646 331 a**	

[a]Volatiles whose concentrations were significantly different ($P \leq 0.05$) among rootstocks are in bold. [b]Calculated retention indices based on a series of *n*-alkanes. [c]Published retention indices on DB-5 column according to the Flavornet database (www.flavornet.org). [d]Odor descriptions according to the Flavornet (http://www.flavornet.org and the University of Florida Citrus Flavor Database (http://www.crec.ifas.ufl.edu/rouseff).

macrophylla rootstocks (Table 3). Thus, the total amount of aroma volatiles in 'Redson' fruits grown on SO rootstock was significantly higher than the amounts in those on Volka and macrophylla rootstocks (Table 3).

In 'Valencia' oranges, we detected a total of 32 volatiles, comprising 5 alcohols, 9 aldehydes, 5 esters, 1 ketone, 7 monoterpenes, and 5 sesquiterpenes (Table 4). We noticed only a few differences in aroma volatiles compositions among fruits grown on various rootstocks: carvone levels were significantly higher in fruits grown on SO than in those on Volka and ×639, and the levels of four terpenes (α-pinene, sabinene, β-pinene, and limonene) and one sesquiterpene (copaene) were significantly higher in fruits on ×639 than in those on SO and Volka rootstocks (Table 4). The total amount of aroma volatiles in 'Valencia' oranges was significantly higher in those on ×639 rootstock than in those on SO and Volka rootstocks, but no significant differences were observed between SO and Volka rootstocks (Table 4).

Effects of Rootstock/Scion Combinations on the Flavor of Citrus Fruits. In addition to biochemical analysis of taste and aroma components, we also examined the effects of rootstock/scion combinations on sensory quality and flavor perception, by means of various preference, descriptive, and discrimination tests. Regarding 'Or' mandarins, it was found that the different rootstocks evaluated (SO, Volka, and US-812) did not have any significant effects on the flavor preferences and flavor profiles of the fruits (Figure 3A,B). Furthermore, in conducting a discrimination test (triangle tests) between 'Or' mandarin fruits grown on SO and those on Volka, we did not detect any significance difference, and of the tasters who perceived the differences among the rootstocks, 47.1% preferred the fruits on

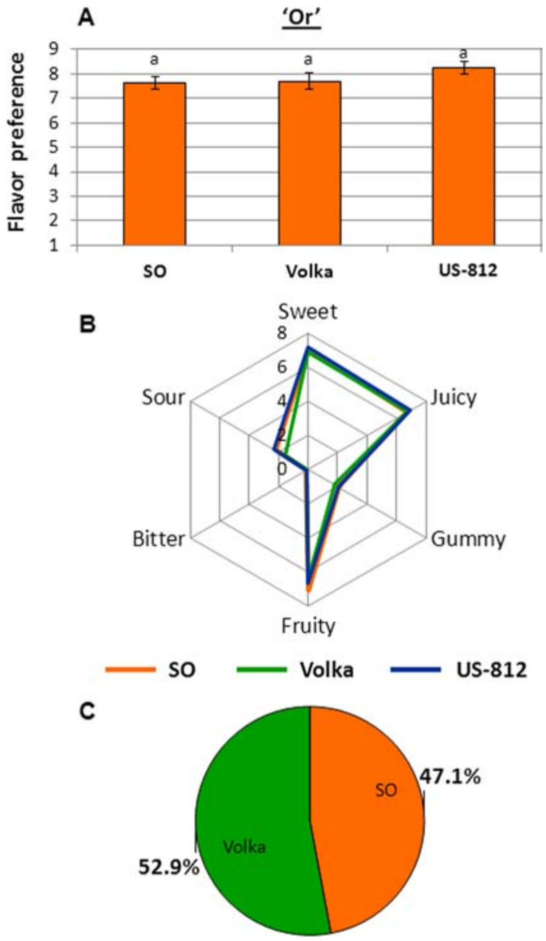

Figure 3. Effects of various rootstocks on the flavor of 'Or' mandarins. (A) Flavor preferences as rated on a nine-point hedonic scale ranging from "very strong dislike" to "very strong like"; (B) descriptive flavor profiles as evaluated by a trained sensory panel; (C) flavor preferences among testers who successfully discriminated between fruits grafted on SO and those grafted on Volka rootstocks. Different letters indicate significant differences at $P \leq 0.05$.

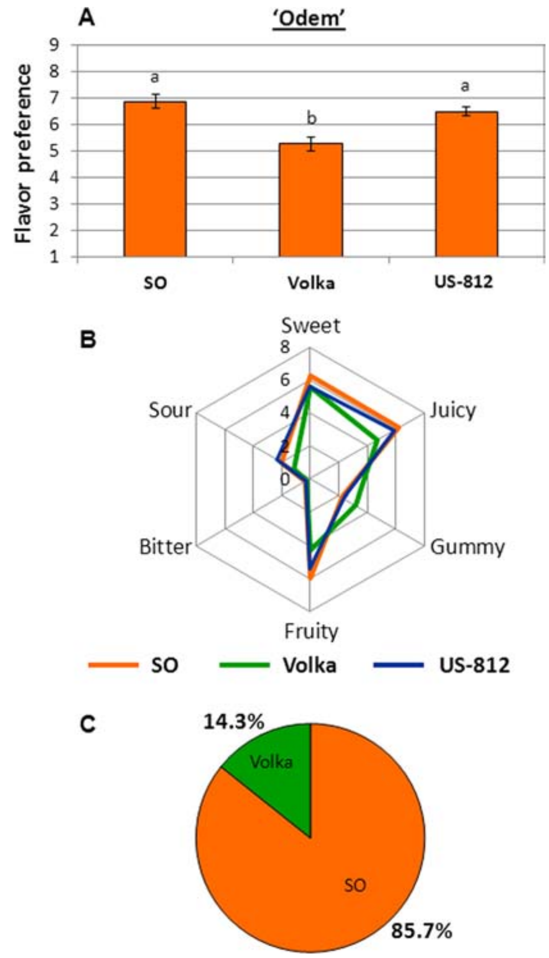

Figure 4. Effects of various rootstocks on the flavor of 'Odem' mandarins. (A) Flavor preferences according to a nine-point hedonic scale ranging from "very strong dislike" to "very strong like"; (B) descriptive flavor profiles as evaluated by a trained sensory panel; (C) flavor preferences among testers who successfully discriminated between fruits grafted on SO and those grafted on Volka rootstocks. Different letters indicate significant differences at $P \leq 0.05$.

SO rootstock, and 52.9% preferred those on Volka rootstock (Figure 3C).

In the case of 'Odem' mandarins, it was clear that the sensory quality of fruits grafted on SO and US-812 rootstocks was preferred to that of fruit on Volka (Figure 4A). Furthermore, descriptive tests conducted by a trained sensory panel revealed that flavor perception of fruits grown on Volka differed from that of those on SO and US-812 rootstocks in being less fruity, juicy and sour, and more gummy (Figure 4B). Indeed, these findings were further confirmed by the results of a discrimination test between 'Odem' mandarins grown on SO and those on Volka: significance differences were found ($P = 0.001$), and of the tasters who correctly identified the differences among the rootstocks, 85.7% preferred the flavor of the fruit grafted on SO rootstock, and only 14.3% preferred the flavor of fruit on Volka rootstock (Figure 4C).

Similarly to the case of 'Odem' mandarins, we also detected notable differences in flavor preferences of the new pummelo × grapefruit hybrid 'Redson' grown on different rootstocks. It was clearly demonstrated that the flavor of 'Redson' fruits on SO was strongly preferred to that of fruit on Volka and macrophylla rootstocks (Figure 5A). According to the descriptive sensory analysis test, it seems that 'Redson' fruits on SO were somewhat more fruity and juicy than those on Volka and macrophylla rootstocks (Figure 5B). Furthermore, fruits grown on Volka were considered less sweet and those on macrophylla somewhat less sour (Figure 5B). In addition, discrimination tests between 'Redson' fruits grown on SO and those on Volka revealed a significant difference ($P \leq 0.001$), and of the tasters who correctly identified the differences among the rootstocks, 88% preferred the flavor of the fruits on SO rootstock, and only 12% preferred the flavor of those on Volka rootstock (Figure 5C).

Finally, as in the case of 'Or' mandarins, we did not detect any differences in sensory quality among 'Valencia' oranges grown on SO, Volka, and ×639 rootstocks, either with a preference test conducted by untrained tasters or with a descriptive test conducted by trained sensory panelists (Figure 6A,B). Furthermore, a discrimination test between 'Valencia' oranges grafted on SO and those on Volka did not reveal any significant differences in fruit flavor preferences (Figure 6C).

■ DISCUSSION

It is known that rootstocks affect TSS and acidity levels in citrus juice and that some rootstocks, such as SO that was tested in the present study, are considered to provide fruits of high

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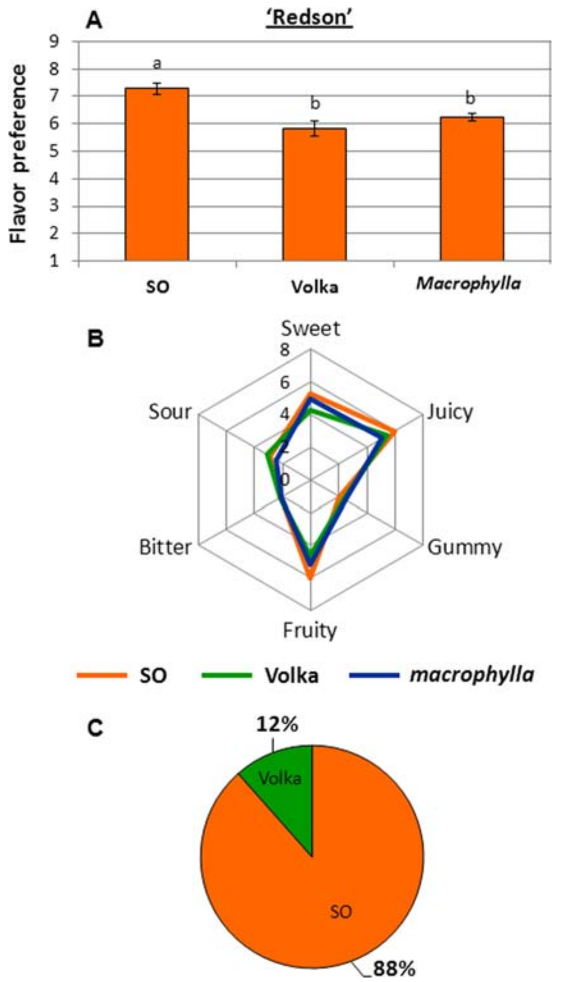

Figure 5. Effects of various rootstocks on the flavor of a pummelo × grapefruit hybrid cv. 'Redson'. (A) Flavor preferences as rated on a nine-point hedonic scale ranging from "very strong dislike" to "very strong like"; (B) descriptive flavor profiles as evaluated by a trained sensory panel; (C) flavor preferences among testers who successfully discriminated between fruits grafted on SO and those grafted on Volka rootstocks. Different letters indicate significant differences at $P \leq 0.05$.

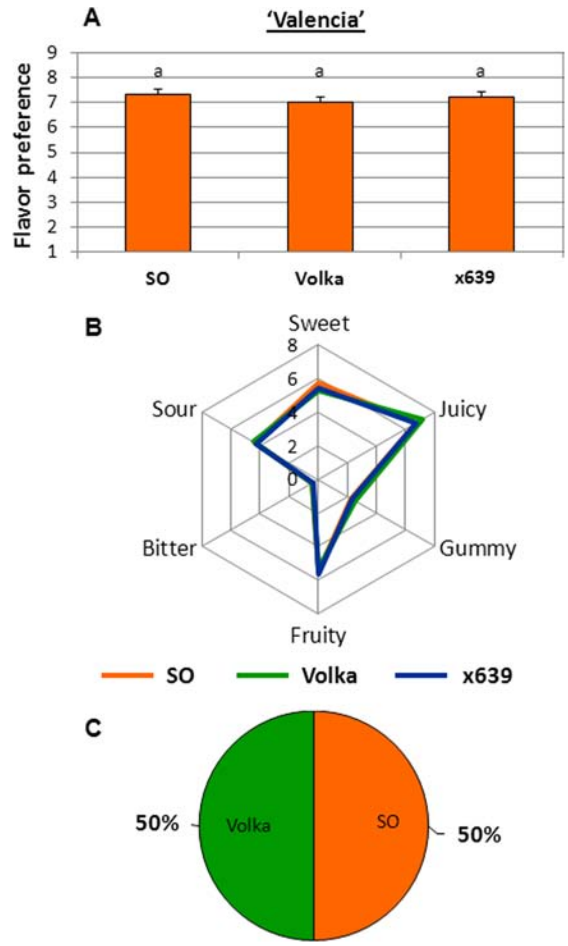

Figure 6. Effects of various rootstocks on the flavor of 'Valencia' oranges. (A) Flavor preferences as rated on a nine-point hedonic scale ranging from "very strong dislike" to "very strong like"; (B) descriptive flavor profiles as evaluated by a trained sensory panel; (C) flavor preferences among testers who successfully discriminated between fruits grafted on SO and those grafted on Volka rootstocks. Different letters indicate significant differences at $P \leq 0.05$.

internal quality, whereas other rootstocks, such as Volka, also tested in the present study, are considered to provide fruits of inferior internal quality.[1−4] However, the particular effects of rootstock/scion combinations on sensory quality and compositions of citrus fruit aroma volatiles have not previously been studied in a systematic and comprehensive manner. In the present study, we examined the effects of various rootstocks, including SO, Volka, and some additional commercially important rootstocks, such as US-812, macrophylla, and ×639, on the sensory quality and the taste and aroma constituents of four citrus varieties comprising two mandarins, a pummelo × grapefruit hybrid, and an orange.

The present results indicate that the effect of rootstocks on the flavor of citrus fruits is a rather complex phenomenon that greatly depends on specific interactions between the rootstock and each particular scion variety. It was observed that the various rootstocks tested did not have any significant effect on flavor perception of 'Or' mandarins or 'Valencia' oranges but significantly affected flavor perception of 'Odem' mandarins and of the pummelo × grapefruit hybrid 'Redson' (Figures 3−6); the flavor of 'Odem' mandarins grown on SO and US-812 was significantly preferred to that of fruits on Volka, and the flavor of 'Redson' fruits grown on SO was significantly preferred to that of those on Volka and macrophylla rootstocks (Figures 4 and 5).

We do not know for sure why the various rootstocks influenced the flavor of 'Odem' and 'Redson' fruits but not that of 'Or' or 'Valencia' fruits. However, we may relate this phenomenon to specific influences of different rootstocks on TSS and acidity levels and aroma volatiles contents of each variety. First, with regard to the effects of different rootstocks on juice TSS and acidity levels of citrus fruits, we found that juice TSS and acidity levels were always somewhat lower in fruits grown on Volka than in those on SO (Figure 2). However, we suggest that these differences may be negligible in terms of sensory perception of varieties that have proper amounts of about 12−13% TSS and 0.9−1.5% acidity, whereas in contrast, the lower TSS and acidity levels that are a consequence of some rootstocks might result in impaired flavor perception in varieties that anyway normally contain relatively low TSS and acidity levels. For example, the decrease in observed TSS levels in 'Redson' fruits from 10% on SO rootstock to just 7.5% on Volka and the decrease in observed acidity levels in 'Odem' mandarins from 0.7% on SO rootstock to just 0.5% on Volka would be expected to result in bland fruits that elicit low sweet and sour sensations, with consequently lower overall flavor preferences (Figures 2, 4, and 5).

Second, with regard to the effects of different rootstocks on levels and composition of aroma volatiles, we detected barely any differences between aroma volatiles levels of 'Or' and 'Valencia' fruits grown on SO and those on Volka but observed significantly lower levels of volatiles, particularly terpenes, terpene alcohols, and aldehydes, in 'Odem' and 'Redson' fruits grown on Volka than in those grown on SO (Tables 1−4). The lower aroma volatile levels of 'Odem' and 'Redson' fruit grown on Volka probably impaired flavor perception; these fruits were perceived as less fruity and achieved overall lower flavor preference scores than those on SO (Figures 4 and 5). For example, 'Odem' and 'Redson' fruits grown on Volka, as compared with SO, had lower levels of linalool, β-pinene, and limonene, which provide floral, piney, and lemon/orange odors, respectively (Tables 2 and 3).

Thus, specific rootstock/scion combinations may affect citrus fruit sensory quality by affecting TSS, acidity, and aroma volatiles levels, and these qualities perhaps may be governed by the level of rootstock/scion compatibility, which obviously affects the translocation of water, nutrients, plant growth regulators, and photosynthetic assimilates through the graft union.[21,22]

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Notes

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