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Four New SuperSour Rootstocks for Improved Production of Sweet Orange in a Huanglongbing Environment

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US SuperSour 2, US SuperSour 3, US SuperSour 4, and US SuperSour 5 are new citrus rootstock cultivars released during 2018 to 2023 (Bowman 2018a, 2018b, 2023a, 2023b) by the Agricultural Research Service (ARS) of the US Department of Agriculture (USDA). The major positive attributes of these new citrus rootstocks are superior field performance with sweet orange scion in huanglongbing (HLB)-endemic regions affected by *Candidatus* Liberibacter asiaticus (*C*Las). Superior performance included superior canopy health and fruit production. All four of these new rootstocks can be easily propagated by stem cuttings or micropropagation, and one of them is also uniformly and efficiently propagated by apomictic seed.

Origin

The four hybrid rootstock selections originated from crosses made at the USDA A.H. Whitmore Foundation Farm by Kim Bowman between 2000 and 2006, and each has distinctly different parentage (Table 1). These hybrids are the product of a new USDA citrus rootstock breeding strategy, termed SuperSour, that has been previously described (Bowman and Joubert 2020; Bowman et al. 2021, 2023). Generally, the SuperSour strategy uses a broad range of parental material that includes either standard sour orange itself (*C. aurantium*) or some combination of the parental species that gave rise to standard sour orange, the mandarin (*C. reticulata*), and pummelo (*C. maxima*). In some progeny, *P. trifoliata* and other species were also included in parentage because of useful rootstock traits. Selection, evaluation, and field testing of these four new rootstocks were planned and conducted by Kim Bowman in collaboration with or support from industry partners, including Florida Citrus Research Foundation and Florida Citrus Research and Development Foundation. Dr. Greg McCollum (USDA ARS, retired) collaborated during the evaluation of fruit quality from the field trials through 2021.

Description

The parentage of two of the new rootstocks, SuperSour 4 and 5, is very similar to the ancestral composition of standard sour orange (*C. maxima* × *C. reticulata*), and leaf traits of these two hybrids are also very similar to those of standard sour orange (Fig. 1, Table 2), without any readily discernable differences. The two other new rootstocks, SuperSour 2 and 3, contain some *P. trifoliata* ancestry and correspondingly exhibit predominate trifoliate leaves that are somewhat unique and may be visually recognizable. It should be noted that many citrus rootstocks and hybrids have similar leaf morphologies, and leaf traits can vary greatly by plant age and growing conditions; therefore, conclusive identification of the new or similar unknown rootstocks should be accomplished using molecular genetic markers. We have previously described one such marker system using simple sequence repeats that is practical to use with citrus rootstocks (Bisi et al. 2020). The unique characteristics of the four new SuperSour rootstocks using that same simple sequence repeats marker system are described in this work (Table 3). The simple sequence repeats markers were also applied to seedlings of these rootstocks and identified the lack of genetic uniformity from seed for two of the new rootstocks (SuperSour 4 and 5), whereas SuperSour 2 exhibited good genetic uniformity among seedlings. Source trees of SuperSour 3 have not yet fruited; therefore, potential uniformity from seed has not been assessed.

Field Performance

Sweet orange tree performance in an HLB-endemic environment has been previously described as generally poor, but it may be improved significantly through the use of tolerant rootstocks (Bowman et al. 2016a, 2016b; Bowman and Albrecht 2020). For the trials reported here, rooted cuttings were used for propagation of each rootstock (Bowman and Albrecht 2017), and the resulting rootstock plants were grafted with 'Valencia' or 'Hamlin' sweet orange scion in a nursery using standard practices (Bowman and Albrecht 2021). When trees reached a suitable size, they were transplanted into the field site at approximately 1 year of age.

Field performance with Valencia scion. Three of the new rootstocks were included in a large replicated trial planted with 'Valencia' sweet orange (*C. sinensis*) scion in St. Lucie County, FL, USA. In this trial, 10 to 12 'Valencia' sweet orange trees on SuperSour 3, 4, and 5 were compared with a similar number of trees on 47 other rootstocks, including the common rootstocks Swingle, standard sour orange, Cleopatra, and Ridge orange, planted in 2014, and using a randomized block statistical design. Swingle and standard sour orange are two of the most common rootstocks used in Florida. A detailed report of rootstock performance during this trial was published (Bowman et al. 2023). Overall tree survival through 2021 was 94%, and tree testing by reverse-transcription polymerase chain reaction revealed that 100% of the trees were infected by *C*Las and 90% were infected by citrus tristeza virus. All surviving trees on each rootstock were used to assess graft compatibility, tree size, tree health, fruit production, and fruit quality.

Graft compatibility. Compatibility was very good for 'Valencia' trees on SuperSour 3, 4, and 5, with a scion-to-rootstock trunk ratio of these combinations of 0.79 to 0.88, indicating a good match of scion and rootstock growth and low likelihood of associated graft abnormalities (Bowman et al. 2023). The scion-to-rootstock trunk ratio of 'Valencia' trees on standard sour orange and 'Swingle' were 0.90 and 0.66, respectively.

Canopy health and size. Canopy health for 'Valencia' on SuperSour 4 and 5 were the two best rootstocks during the trial over multiple evaluations during 2020–21, with average scores of 4.58 and 4.44, respectively, based on a scale of 1 to 5 (Bowman et al. 2023). 'Valencia' on SuperSour 3, standard sour orange, 'Swingle' citrumelo, 'Cleopatra' mandarin, and 'Ridge' orange had scores of 4.00, 4.05, 3.95, 3.67, and 3.17, respectively. Canopy health score continued to be good for trees on SuperSour 3, 4, and 5 during 2022–23, and as good or better than that on the standard rootstocks (Fig. 2). An analysis of tree images obtained using an unmanned aerial vehicle (Aerobotics US, Inc., Fresno, CA, USA) provided similar relative canopy health assessments in December 2022, and values of the normalized difference vegetation index were compared (Fig. 2). Canopy size of 'Valencia' trees on SuperSour 3, 4, and 5 were all in the large category and similar to trees on standard sour orange.

Fruit yield and premature drop. During the Picos 2014 trial, the cumulative fruit yield

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Table 1. Parentage and release date of the rootstocks.

Rootstock	Parentage[i]	Release date
Cleopatra	*Citrus reticulata*	
Ridge	*Citrus sinensis*	
Standard sour orange	*Citrus aurantium*	
Swingle	*Citrus paradisi* × *Poncirus trifoliata*	
US SuperSour 2	*P. trifoliata* 'Benecke' × (*C. aurantium* 'Chinotto' × *Citrus ichangensis*)	2018
US SuperSour 3	*C. reticulata* 'Sunki' × US-802 (*Citrus maxima* × *P. trifoliata*)	2018
US SuperSour 4	*C. maxima* 'Hirado' × *C. reticulata* 'Cleopatra'	2023
US SuperSour 5	*C. maxima* 'Mato' × *C. reticulata* 'Shekwasha'	2023

[i] The parent identified as US-802 was released by the US Department of Agriculture as a promising new rootstock in 2007. The parent identified as *C. maxima* 'Hirado' is a Florida selection from seedlings of 'Hirado Buntan'. The parent identified as *C. maxima* 'Mato' is a Florida selection from seedlings of 'Mato Buntan'.

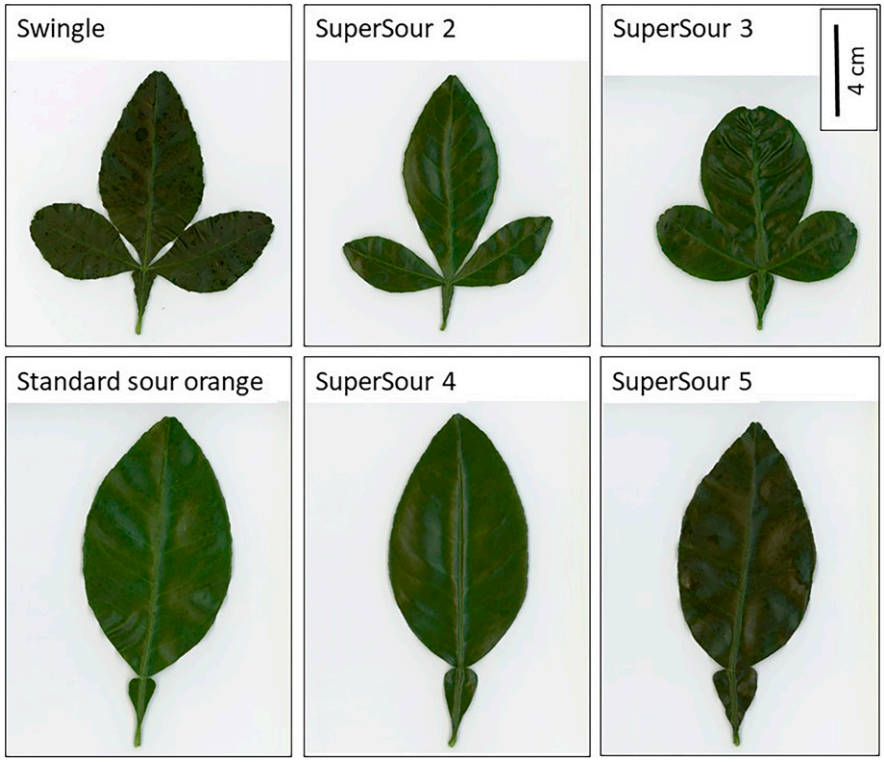

Fig. 1. Greenhouse-grown leaves of the six rootstock clones.

of 'Valencia' trees on US SuperSour 4 over the first four harvests (2018–21) was the largest yield of any rootstock in the trial, at 56.8 kg per tree (Bowman et al. 2023). In comparison, 'Valencia' on the other rootstocks SuperSour 5, SuperSour 3, standard sour orange, 'Swingle', 'Cleopatra', and 'Ridge' had cumulative yields of 46.5 kg, 43.3 kg, 37.3 kg, 34.5 kg, 17.9 kg, and 11.8 kg fruit per tree, respectively. These were 65%, 35%, and 26% fruit yield increases for 'Valencia' sweet orange trees on SuperSour 4, SuperSour 5, and SuperSour 3 rootstocks, respectively, compared with 'Swingle', which is one of the most popular rootstocks for sweet orange in Florida. Cumulative fruit yield continued to be superior for the three new rootstocks during the 2022–23 seasons (Fig. 3). Preharvest fruit drop is a major problem for the Florida sweet orange crop when trees are infected with *C*Las, with a large portion of the crop dropping to the ground and spoiling before quality is suitable for commercial harvest. Trees of 'Valencia' on SuperSour 4 had the second lowest preharvest fruit drop of any rootstock in the trial in 2021, with 28% of the crop dropping during the 4 weeks preceding harvest in 2021 (Bowman et al. 2023). In comparison, 'Valencia' on SuperSour 5, SuperSour 3, standard sour orange, 'Swingle', 'Cleopatra', and 'Ridge' had preharvest fruit drops of 39%, 37%, 50%, 44%, 61%, and 69%, respectively (Fig. 3).

Fruit quality. During the 2020–23 harvest seasons, 'Valencia' fruit quality on SuperSour 3, SuperSour 4, and SuperSour 5 rootstocks were similar to that on standard sour orange and 'Swingle' at harvest in early March. Fruit quality of juice oranges is primarily assessed as the weight of total soluble solids (TSS) per fruit weight. At harvest time during 2020–23, the TSS (kg) per metric ton (MT) of fruit for SuperSour 3, SuperSour 4, and SuperSour 5 were 42.8, 39,3, and 38.7, respectively, whereas the kg/MT values for standard sour orange and 'Swingle' were 37.1 and 41.8, respectively (Fig. 4). Although of lesser importance than TSS, juice color is of some importance to sweet orange fruit quality. Rootstock had a small but significant effect on 'Valencia' juice color, with 'Swingle' providing the best juice color, standard sour orange providing the worst, and SuperSour 3, SuperSour 4, and SuperSour 5 having juice color numbers intermediate between the two standard rootstocks (Fig. 4).

Production per hectare. At the trial planting density, US SuperSour 4 was the best rootstock during the trial of fruit TSS per hectare per season, at 440 kg TSS, during the 2020–21 harvest season (Bowman et al. 2023). For comparison, trees on SuperSour 5, SuperSour 3, standard sour orange, 'Swingle', 'Cleopatra', and 'Ridge' rootstocks produced 399 kg, 314 kg, 343 kg, 274 kg, 165 kg, and 69 kg TSS per hectare per season, respectively. These are 61%, 46%, and 15% increases in TSS production per hectare for SuperSour 4, SuperSour 5, and SuperSour 3 compared with 'Swingle' rootstock.

Table 2. Leaf traits of greenhouse-grown plants of 'Swingle', standard sour orange, and SuperSours 2, 3, 4, and 5.

Trait	Swingle	SuperSour 2	SuperSour 3	SuperSour 4	SuperSour 5	Standard sour orange
Unifoliate leaves (%)	0	0	5	100	100	100
Bifoliate leaves (%)	0	0	15	0	0	0
Trifoliate leaves (%)	100	100	80	0	0	0
Main leaf blade length (mm)	83.3 b	81.4 bc	73.5 c	102.7 a	99.9 a	97.4 a
Main leaf blade width (mm)	42.2 b	35.1 c	45.1 b	56.0 a	55.0 a	53.2 a
Left/right blade length (mm)	49.1 a	41.6 b	41.1 b			
Left/right blade width (mm)	26.6 a	18.3 c	22.1 b			
Petiole length (mm)	26.3 c	18.9 e	22.6 d	32.9 a	30.4 ab	27.7 bc
Petiole width (mm)	8.0 c	4.8 d	6.6 cd	14.9 a	13.0 ab	11.5 b

Analysis of variance (ANOVA) comparisons within rows for leaf dimensions were all significant at $P < 0.000001$.
Mean groups for the significant ANOVA within rows were determined by Tukey test using $P < 0.05$.

Table 3. Amplified alleles of simple sequence repeat markers of 'Swingle', standard sour orange, and SuperSours 2, 3, 4, and 5, and trueness-to-type from seed.

SSR primer	Swingle	SuperSour 2	SuperSour 3	SuperSour 4	SuperSour 5	Standard sour orange
M165	214, 234	215, 226	215, 235	215, 220	206, 215	215, 220
M172	249, 252	253, 264	247, 255	248, 272	252, 272	247, 255
M13	128, 142	128, 133	128, 133	131, 143	130, 143	128, 141
M156	179, 182	179, 185	179, 185	182, 191	182, 191	179, 188
M21	361, 365	364, 373	361, 373	362, 374	365, 374	364, 374
M50	143, 161	143, 149	143, 149	150, 156	156	149, 161
M112	248, 257	248, 251	248, 251	248, 251	248, 251	248, 251
M126	170, 185	185	171, 186	171, 186	171, 186	171, 186
M157	233, 236	242	236, 242	233, 242	233, 242	233, 242
M163	232, 250	232, 250	232, 250	250	250	241, 256
Seedling trueness-to-type (%)	100	95	ND	0	0	90

ND = no data; SSR = simple sequence repeat.

Field performance with Hamlin scion. All four of the new SuperSour rootstocks were included in replicated trials with 'Hamlin' sweet orange (*C. sinensis*) in Lake County, FL, USA, in an area where the soil is Astatula fine sand with a pH of 6.2 and good natural drainage. Although all the trials were at the same research site, the different SuperSour rootstocks were in trials planted in different years and, correspondingly, comparisons were made with standard rootstocks in each trial. At this site, field trees exhibited widespread symptoms of HLB disease and are known to be uniformly infected with *C*Las (similar to the St. Lucie County Valencia trial) by the time the trees are 3 to 4 years of age.

SuperSour 2 rootstock was evaluated during a replicated Lake County trial with 'Hamlin' scion planted in 2011 and compared with standard rootstocks for performance through 2017. In this trial, the cumulative yield (2015–17) on SuperSour 2 rootstock was 46 kg fruit per tree; this yield was 53%, 92%, 170%, and 171% higher than those of trees over the same time period on 'Swingle', standard sour orange, 'Cleopatra', and 'Ridge' rootstocks, respectively. Regarding the rootstock effect on fruit quality, 'Hamlin' on SuperSour 2 rootstock during the 2016 season yielded 48.5 kg TSS per MT of fruit, whereas trees on 'Swingle' and standard sour orange yielded 44.4 kg and 42.1 kg TSS per MT, respectively.

SuperSour 3 rootstock was evaluated during a replicated Lake Country trial with 'Hamlin' scion planted in 2015 and compared with standard rootstocks for performance during 2018–22. During this trial, 'Hamlin' on SuperSour 3 had a cumulative yield of 226 fruit per tree; this yield was 1%, 17%, and 31% higher than those of trees on 'Swingle', standard sour orange, and 'Cleopatra', respectively. Regarding the rootstock effect on fruit quality, 'Hamlin' on SuperSour 3 rootstock during the 2021 season yielded 46.4 kg TSS per MT of fruit, whereas trees on 'Swingle', standard sour orange, and 'Cleopatra' yielded 43.2 kg, 44.6 kg, and 45.0 kg TSS per MT of fruit.

SuperSour 4 rootstock was evaluated during a replicated Lake County trial with 'Hamlin' scion planted in 2018 and compared with standard rootstocks for performance during 2020–22. During this trial, 'Hamlin' on SuperSour 4 had a cumulative yield of 104 fruit per tree; this yield was 37%, 51%, and 117% higher than those of trees on 'Swingle', standard sour orange, and 'Cleopatra', respectively.

SuperSour 5 rootstock was evaluated during a replicated Lake Country trial with 'Hamlin' scion planted in 2017 and compared with standard rootstock performance during 2020–22. During this trial, 'Hamlin' on SuperSour 5 had a cumulative yield of 143 fruit per tree; this yield was 43%, 44%, and 49% higher than those of trees on 'Swingle', standard sour orange, and 'Cleopatra', respectively.

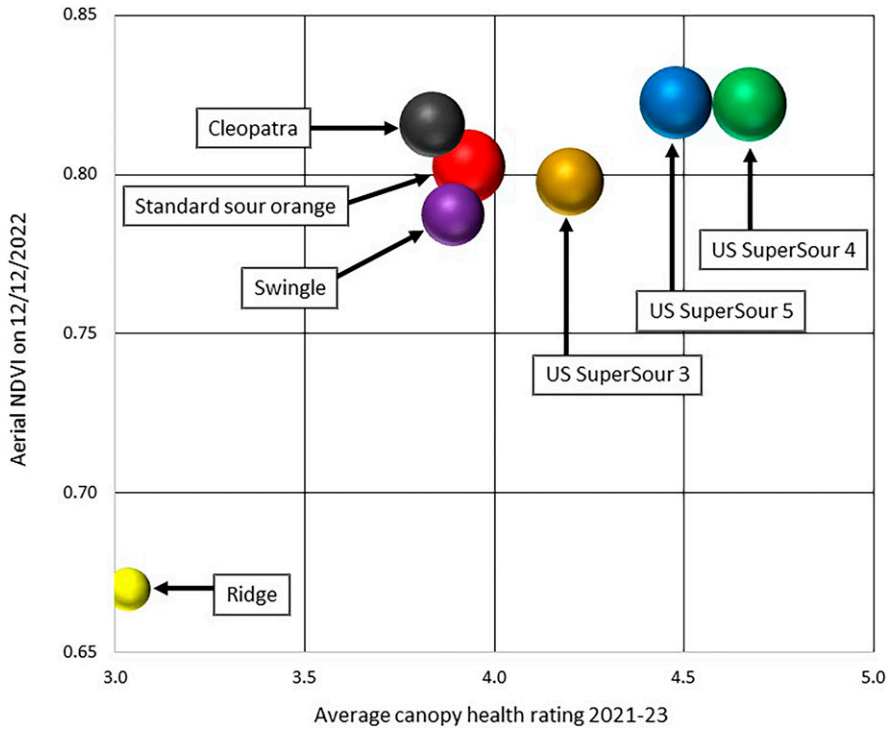

Fig. 2. Aerial normalized difference vegetation index (NDVI) (Dec 2022), canopy health rating (2021–23), and canopy volume (balloon size) of trees in the Picos 2014 trial of US SuperSour 3, 4, and 5 and the four standard rootstocks. Analysis of variance: aerial NDVI, p>F = 0.00006; canopy health rating, p>F = <0.00001; canopy volume, p>F = <0.00001.

Cultivation

The four new SuperSour rootstocks are used and cultivated in the same way as other common rootstocks for citrus trees. After rootstock plants are propagated in a citrus nursery by cuttings, micropropagation, or apomictic seed, a suitable citrus scion cultivar is grafted on top. Then, nursery trees are planted in the field and grown by standard methods for fruit production over a series of years. The four SuperSour rootstocks described here provided performance superior to that of other standard rootstock cultivars while growing under identical conditions.

Availability

All four of these new SuperSour rootstocks have been planted in additional trials to further assess relative performance among each other, and with additional rootstocks, under a range of different production conditions and scion cultivars. They have been released now because of great urgency to provide the Florida citrus industry with new rootstocks that will increase production and profitability in the HLB-endemic environment. Source plant material for SuperSour 2, SuperSour 3, SuperSour

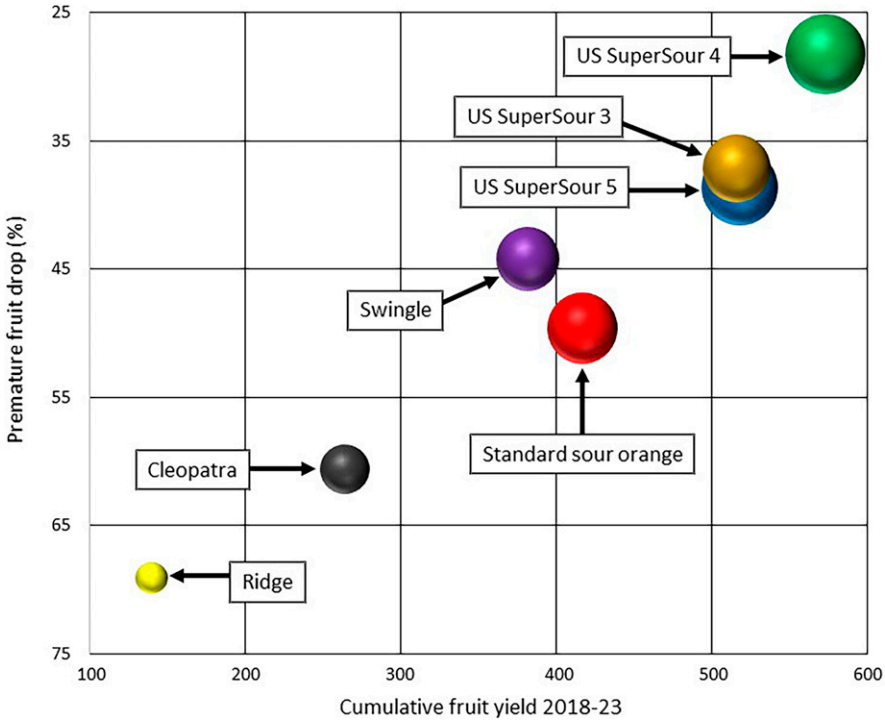

Fig. 3. Premature fruit drop (2021), cumulative fruit yield per tree (2018–23), and soluble solids per hectare per season (balloon size at 640 trees/hectare) for trees in the Picos 2014 trial of US SuperSour 3, 4, and 5 and the four standard rootstocks. Analysis of variance: premature fruit drop, p>F = 0.00017; cumulative fruit yield, p>F = <0.00001; soluble solids per hectare per season, p>F = <0.00001.

4, and SuperSour 5 rootstocks is available from the Florida Bureau of Citrus Budwood Registration clean budwood program (3027 Lake Alfred Road, Highway 17, Winter Haven, FL 33881, USA) and will be distributed from the Chiefland Budwood Foundation (9870 NW 42nd Court, Chiefland, FL 32626, USA) according to Florida Department of Agriculture and Consumer Services regulations. Plant tissue of all four new SuperSour rootstocks for research as well as additional information regarding the new rootstocks may be obtained from the author (kim.bowman@usda.gov). Genetic material of this release will be deposited in the National Plant Germplasm System, where it will be available for research purposes, including development and commercialization of new cultivars. Appropriate recognition should be made if this germplasm contributes to the development of a new breeding line or cultivar.

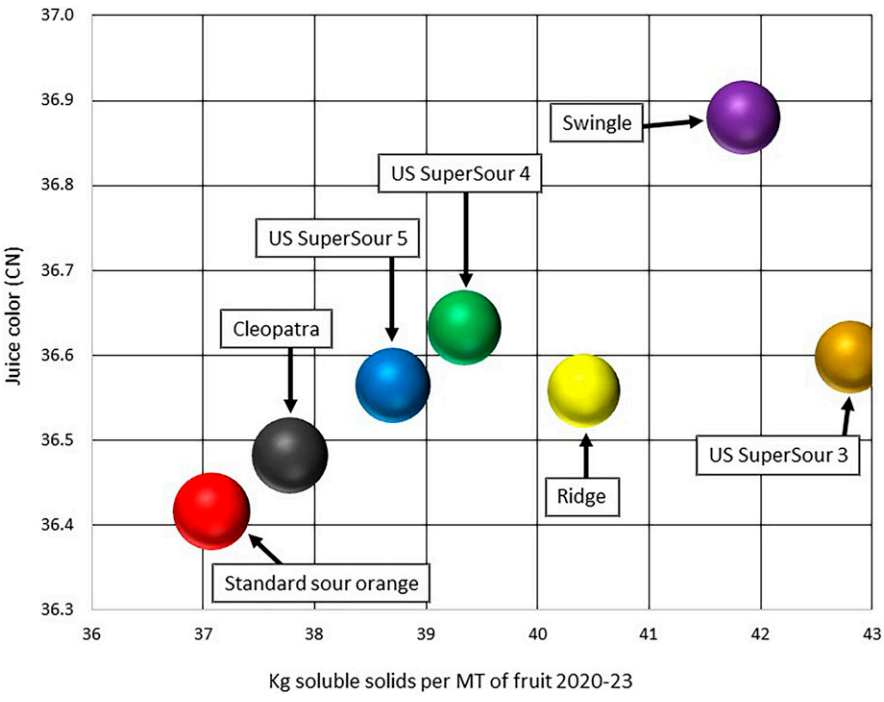

Fig. 4. Juice color, kg soluble solids per MT of fruit, and juice total soluble solids-to-acid ratio (balloon size) for trees in the Picos 2014 trial at harvest 2020–23 of US SuperSour 3, 4, and 5 and the four standard rootstocks. Analysis of variance: juice color, p>F = 0.00690; kg soluble solids per MT, p>F = <0.00001; soluble solids-to-acid ratio, p>F = 0.01324.

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