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Effects of Oxytetracycline Trunk Injection on Fruit and Juice Quality of Florida-grown 'OLL-8' Sweet Orange Planted on Different Rootstocks

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Abstract. **The devastating bacterial citrus disease huanglongbing (HLB) has led to a nearly 90% decline in citrus fruit production in the State of Florida since its first identification nearly two decades ago. Most of the Florida citrus industry is comprised of sweet orange [*Citrus sinensis* (L.) Osbeck] primarily used for juice processing. All known commercial sweet orange varieties are susceptible to HLB. Of the sweet orange varieties released by University of Florida Institute of Food and Agricultural Sciences, 'OLL' lines have shown some degree of tolerance to HLB in field trials. In this experiment, we evaluated the effects of rootstock and oxytetracycline (OTC) trunk injection on the fruit and juice quality of the 'OLL-8' sweet orange scion. Of the rootstocks evaluated, three were industry standards ('US-942', 'US-897', and 'Swingle') and three were UF rootstocks ('UFR-2', 'UFR-4', and 'UFR-6'), and each rootstock–scion combination had trees that were treated with OTC or were left untreated. Data were collected on juice quality attributes including titratable acidity, total soluble solids, sugar:acid ratio, pounds-solids per box, and juice color, as well as mass, diameter, and total yield. The data were analyzed to determine statistically significant differences among the different rootstock–'OLL-8' combinations, and the treatments were evaluated. The OTC-treated trees had a significantly larger mean fruit diameter, higher mean ratio, and lower premature fruit drop than nontreated trees. Additionally, rootstock selection in conjunction with the 'OLL-8' scion significantly affected each of the fruit and juice quality characteristics evaluated. The results indicated that rootstock selection and OTC trunk injection can significantly improve fruit and juice quality of the 'OLL-8' sweet orange scion.**

Citrus cultivation has had an enormous impact on Florida's economy. Following the first identification of huanglongbing (HLB) in Florida in 2005, there has been a drastic decline in acreage dedicated to citrus and boxes of fruit produced, as well as fruit and juice quality. During the 1997–98 harvest season, ~820,000 citrus acres were cultivated with 304 million boxes of citrus produced, whereas in the 2022–23 season, only 18.1 million boxes were produced across 340,000 acres [US Department of Agriculture National Agricultural Statistics Service (USDA NASS) 2000, 2024]. During the 1997–98 season, on-tree citrus crop value was reported to be $1.02 billion (USDA NASS 2000) compared with the 2022–23 season, during which the value was recorded at only $208 million (USDA NASS 2024). Most of the citrus production in Florida is comprised of sweet orange varieties [*Citrus sinensis* (L.) Osbeck], with most fruit designated for juice processing. During the 2022–23 season, nearly 83% of all sweet orange production in Florida was processed as juice, whereas during the 1997–98 season, this value was higher at 95% (USDA NASS 2024, 2000). HLB-affected trees have been associated with premature fruit drop, and symptomatic fruit have been associated with lower total soluble solids (TSS), higher titratable acidity (TA), and lower ratio (TSS:TA) (Bassanezi et al. 2009, 2011; Dagulo et al. 2010).

Many methods have been explored to suppress or control the spread of HLB across Florida and other growing regions. The bacteria associated with HLB in Florida is proposed to be *Candidatus* Liberibacter asiaticus (*C*Las), and to date there is no method to obtain a pure culture in vitro, which limits the understanding of pathogen–host interactions and important physiological characteristics that may aid in disease control (Gottwald 2010). The *C*Las bacteria is transmitted by the Asian citrus psyllid (Pelz-Stelinski et al. 2010) or by grafting (Lopes et al. 2009). Pesticide sprays have been used in attempts to control the spread of HLB; however, in areas such as Florida, where HLB is endemic, this control method may not benefit mature groves where infection is already present (Bassanezi et al. 2013). Similarly, sprays must be conducted multiple times throughout the year in accordance with flush periods for citrus trees (Bassanezi et al. 2020) and the Asian citrus psyllid life cycle. Other methods such as increased micronutrient applications (Zambon et al. 2019), infected tree removal, increased planting density (Moreira et al. 2019) and foliar spray applications of plant growth regulators such as gibberellic acid have been explored (Albrigo and Stover 2015), yet their efficacy varies among studies.

Oxytetracycline (OTC) was discovered in 1950 and was formerly named "terramycin" (Finlay et al. 1950). OTC is classified as a naturally produced polyketide with a characteristic aromatic ring and acts as a bacteriostatic compound. This compound binds the 30 S ribosomal subunit and prevents bacterial protein synthesis (Chopra and Roberts 2001). OTC is a broad-spectrum antibiotic and is widely used to treat bacterial infections including both gram-negative and gram-positive species. Often, OTC is administered in large-scale farm operations such as in cattle or pig farming to control and prevent respiratory and intestinal infections (Pilloud 1973).

In 2016, the US Environmental Protection Agency (EPA) approved the use of OTC and streptomycin foliar sprays for use in Florida citrus groves in attempt to suppress and control HLB. However, only trace amounts of OTC entered citrus leaf tissue and, as a result, failed to meet the minimum inhibitory concentration determined by Killiny et al. (2020). In late 2022, the US EPA approved the delivery of OTC via trunk injections, which produces higher OTC concentrations in leaves and other tissues compared with foliar sprays (Aubert and Bové 1980; Killiny et al. 2020; Li et al. 2019; Vincent et al. 2022). Results have varied among studies and varieties; however, this method of control has been reported to show efficacy in mitigating HLB symptoms. Archer et al. (2023) demonstrated some improvement of HLB symptoms in 'Hamlin' and 'Duncan', while fruit drop was reduced in the two varieties, as well as 'Valencia'. Hu and Wang (2016) reported that *C*Las populations were significantly lower in OTC-injected trees 28 d postinjection compared with control trees.

Rootstock selection in citrus crops can significantly affect outcomes regarding yield, disease resistance and tolerance, vigor, and numerous other factors. For example, rootstocks derived from *Poncirus trifoliata* can confer resistance to *Phytophthora*; 'Sour Orange' performs well under high salinity conditions; 'X-639' produces trees of a larger size compared with 'Flying Dragon', which produces small trees (Castle et al. 2019). Therefore, rootstock selection plays a pivotal role in optimizing citrus production practices.

Some of the most widely used rootstocks in Florida include 'US-942', 'Swingle', and 'US-897'. Rootstocks 'US-942' and 'Swingle' were included as two of the five most propagated rootstocks in 2021 (Florida Department of Agriculture and Consumer Services 2021). Other rootstocks included in this report were 'Kuharske', 'X-639', and 'Sour Orange'. Trees grown on 'US-897' typically result in relatively smaller trees, ideal for high-density plantings; trees on 'US-942' produce trees of small to intermediate size; trees on 'Swingle' result in intermediate sizing. Each of these rootstocks can result in relatively different yields; 'US-942' can produce a high yield, 'Swingle' can produce an intermediate yield, and 'US-897' typically produces the lowest yield of the three (Castle et al. 2019). The 'US-942' rootstock was derived from a cross between *Citrus reticulata* 'Sunki' and *P. trifoliata* 'Flying Dragon'; the 'US-897' rootstock was derived from a cross between *C. reticulata* 'Cleopatra' and *P. trifoliata* 'Flying Dragon'; and the 'Swingle' rootstock was derived from a cross between *Citrus paradisi* and *P. trifoliata*. Despite these commercial rootstocks sharing a common parent, their performance and characteristics vary significantly.

The 'US-942' rootstock was commercially released by the USDA in 2010 and has predominated as one of the top rootstocks used in Florida since. Bowman et al. (2016b) reported that among 17 rootstock cultivars, 'Swingle' and 'US-897' included, trees on 'US-942' rootstocks produced the highest cumulative yield across four seasons of data collection.

The 'US-897' rootstock was commercially released by the USDA in 2007 and is thought to have some level of tolerance to HLB symptoms. Albrecht and Bowman (2011) reported that naturally infected, grafted 'US-897' trees showed few foliar symptoms associated with HLB, although testing positive for *C*Las.

The 'Swingle' rootstock was commercially released in the 1970s and has predominated as one of the top propagated rootstocks in Florida and other citrus producing regions. 'Swingle' is known for its cold tolerance and resistance to pathogens such as *Phytophthora*.

In comparison with the previously mentioned diploid rootstocks, the University of Florida Institute of Food and Agricultural Sciences (UF-IFAS) has released many tetraploid hybrid rootstocks. Tetraploid rootstocks can be associated with reduced tree size and increased stress tolerance (Grosser et al. 2015). For example, 'UFR-2', 'UFR-4', and 'UFR-6' rootstocks are tolerant to *Phytophthora*. The 'UFR-2' and 'UFR-4' rootstocks are allotetraploid hybrids of [(*C. reticulata* × *C. paradisi*) + *C. grandis*] × [*C. reticulata* + *P. trifoliata*]. The 'UFR-6' rootstock was derived from protoplast fusion using *C. reticulata* as the embryogenic suspension culture parent and *P. trifoliata* as the leaf parent. The Florida Citrus Rootstock Selection Guide, 4th edition (Castle et al. 2019) categorizes these three rootstocks as having low to intermediate HLB incidence in the field.

The objective of this experiment was to determine the effects of rootstock selection and OTC trunk injection on 'OLL-8' fruit and juice quality and yields. Three rootstocks released by UF-IFAS and three commercial industry standard rootstocks were evaluated for potential effects on fruit and juice quality and yields in conjunction with the OTC trunk injections.

Materials and Methods

Plant material and field location. The trees used in this experiment were located at the property of a private grower in Haines City, FL, USA (lat. 28°08′43″N, long. 81°42′20″W). The trees were planted 2.44 m apart for in-row spacing and 6.71 m apart for between-row spacing. This resulted in a planting density of 247 trees per acre and 611 trees per hectare. For each rootstock–scion combination, all trees were mature. Each tree was composed of an 'OLL-8' sweet orange scion grafted onto one of six different rootstocks. The 'OLL-8' sweet orange scion was released by the plant breeding team at UF-IFAS and was available for commercial use in 2015. The 'OLL-8' scion was developed using advanced tissue culture techniques. Evaluated in this study were three UF-IFAS released rootstocks, 'UFR-2', 'UFR-4', and 'UFR-6', and three commercial industry standard rootstocks, 'US-942', 'US-897', and 'Swingle'. Each of the rootstock–scion combinations were over 10 years of age, with the exception being 'US-942' which was 5 years of age at the start of the experiment. There were 30 data trees for each of the rootstock–scion combinations; the exception to this was 'US-897', which had 20 data trees. The trees were randomly planted in blocks of 10 trees. The OTC treatment was performed in May 2023 with the Rectify (95% oxytetracycline hydrochloride; AgroSource, Inc., Sebring, FL, USA) solution at 7500 ppm dissolved in 100 mL of tap water and mixed with muriatic acid per product label instructions. The OTC-injected trees were harvested in the second year of the study (the 2023–24 season). For each block, 6 of the 10 trees received OTC trunk injections; these trees were on the perimeter (three on either side). Samples for the non-OTC treatment were collected from the 4 middle trees in each group of 10 trees for each rootstock–scion combination with the exception being groups of 'US-897', for which the 6 middle trees were sampled. There was a total sample size of 12 (n = 12) for the non-OTC treatment, while OTC treatment had a sample size of 18 (n = 18). The exception to this was the 'US-897' rootstock, for which the OTC treatment had a sample size of 8 (n = 8).

Sample and data collection. For the first season of data collection (where there were no trunk injections), trees were harvested three times during the season. Half-bushel (∼9 kg) whole-fruit samples from each data tree were randomly selected throughout the canopy at the first two time points. The first time point occurred on 27 Feb 2023, and the second time point was 21 Mar 2023. For the third and final time point of this harvest season, each tree was stripped of all remaining fruit to obtain metrics on fruit and juice quality and yields. The third harvest occurred on 5 Apr 2023.

Similarly, during year 2 of the study, the data trees were harvested three times. The samples from each of the data trees were collected on 25 Jan 2024. Additionally, during this first time point, three randomly selected trees per rootstock–scion combination per treatment were chosen for whole-tree harvest, in which the tree was stripped of all fruit. The harvest for the second time point was 1 Mar 2024 and consisted of half-bushel samples to determine fruit and juice quality. The third and final harvest was on 20 Mar 2024, when three trees per rootstock–scion combination per treatment were selected for a whole-tree harvest, in which all remaining fruit were stripped from the tree to determine fruit yield and quality. Harvesting timing for both harvest seasons was based on grower instructions and based on tree phenology, with year 2 fruit appearing to have an earlier maturity date compared with year 1 fruit.

Individual fruit weight and diameter. Individual fruits were weighed and measured at the University of Florida Citrus Research and Education Center with the Spectrim single-lane sorter 1-01-01A (Tomra/Compac, Visalia, CA, USA). Fruit weight was measured in grams. For fruit size, the major and minor diameters of each individual fruit were measured in millimeters to obtain the average diameter of each fruit.

Juice sample processing. Juice samples were obtained with a three-head pinpoint extractor 67-257 (JBT, Food Tech Division, Lakeland, FL, USA). Total fruit weight and juice weight of each sample was measured in kilograms and recorded by the previously mentioned Spectrim single-lane sorter 1-01-01A (Tomra/Compac).

Juice quality testing. Juice quality attributes were measured with the Brix Acid Unit

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Automation System (Florida Department of Agriculture and Consumer Services, Division of Fruits and Vegetables, Tallahassee, FL, USA), and included the percentage of TSS and the percentage of TA in citric acid equivalents. Software included in this system calculated the ratio of TSS to TA and pounds-solids per box. For each of these variables, the data collected from the two final harvest dates in each year were used to represent fruit reaching maturity in the HLB environment. For each juice sample, ~9 kg of fruit were needed to produce roughly 3.8 L of juice for processing. Pounds-solids per box values were calculated as follows:

$$\text{Pounds-solids per box} = \frac{\text{Juice weight}}{\text{Sample weight}} \times \text{90-pound box of fruit} \times \text{TSS} \quad [1]$$

Juice color. Approximately 40 to 50 mL of juice were placed into a 25-× 150-mm glass culture tube. The sample was placed into a calibrated X-Rite Ci7520 spectrophotometer (X-Rite, Grand Rapids, MI, USA) to measure the juice color score.

The color number was converted to a color score by the Color iQC software included with the spectrophotometer, based on standards set by the US Department of Agriculture in 1983 with a spectral range of 360 to 750 nm with 20-nm wavelength intervals. The formula for calculating the juice color number was:

$$\text{Color number} = 14.5 \times (3.15X/Y - Z/Y + 3.9/Y) - 2.6 \quad [2]$$

where X, Y, and Z corresponded to red, green, and blue values based on the RGB color model, respectively.

Total fruit yield. For 2022–23 sampling, all collected fruit were sized, sorted, and juiced. Cumulative fruit and juice yields per tree were summed from each individual harvest date. Total fruit and juice yields were collected for 12 replicates per rootstock–scion combination and were measured in kilograms. Fruit drop was measured by counting individual fruits surrounding each tree in Jan 2024 and Mar 2024.

For 2023–24 sampling, six trees of each rootstock–scion combination and treatment underwent a full, whole-tree harvest. Additionally, for each replicate per rootstock–scion combination per treatment, total fruit count was recorded by stripping all fruit from the tree and counting the fruit.

Pounds-solids per hectare was determined with the following formula:

$$\text{Pounds-solids per hectare} = \frac{\text{Juice weight}}{\text{Sample weight}} \times \text{TSS} \times \text{Total fruit weight (lbs)} \times 611\frac{\text{trees}}{\text{hectare}} \quad [3]$$

Pounds-solids per acre was determined with the following formula:

$$\text{Pounds-solids per acre} = \frac{\text{Juice weight}}{\text{Sample weight}} \times \text{TSS} \times \text{Total fruit weight (lbs)} \times 247\frac{\text{trees}}{\text{acre}} \quad [4]$$

Statistical analysis. Analysis of variance was performed using Minitab Statistic Software, version 21.1.1 (Minitab LLC, State College, PA, USA) with an experiment-wise error rate of $\alpha = 0.05$, along with Tukey's honestly significant difference test for post hoc multiple comparisons among sample means. Statistical interaction terms were included in analysis of variance models (general linear model and one-way ANOVA) to determine statistical interactions among rootstock-scion combination and OTC treatment. Regression analysis was performed to determine potential relationships between variable outcomes. Correlation coefficients at $\alpha = 0.05$ were used to determine the strength and direction of relationships among the variables evaluated.

Results

Individual fruit weight and diameter

Individual fruit weight. There was evidence for statistically significant differences among rootstocks for the 2022–23 season for mean fruit weight (Table 1; $P < 0.001$). 'OLL-8' sweet orange on 'US-942' rootstock produced the heaviest mean individual fruit weight of 197.2 g, which was significantly different from all other rootstock–'OLL-8' combinations evaluated in this study. The least heavy mean individual fruit weight of 158.6 g was produced by 'OLL-8' on 'UFR-4' rootstock. Similar results were produced when analyzing rootstock–'OLL-8' combination performance across both seasons ($P < 0.001$); however, when nontreated trees solely from the 2023–24 season were analyzed, there were no significant differences among rootstocks for mean fruit weight ($P = 0.055$). When including the OTC-treated trees in the 2023–24 rootstock analysis, trees with 'US-942' rootstock had a mean fruit weight that was significantly higher than trees with 'UFR-4', 'UFR-2', and 'Swingle' rootstocks ($P = 0.002$).

There was evidence for statistically significant differences in mean individual fruit weight for 'OLL-8' sweet orange among the treatments and rootstocks evaluated for the 2023–24 season (Table 2; $P < 0.001$). The highest mean individual fruit weight was produced by OTC-treated trees with 'US-942' rootstock (186.6 g), and the lowest mean was from nontreated trees with 'UFR-4' rootstock (153.5 g). Each of the rootstocks produced a marginally higher mean fruit weight when treated with OTC, although none of these differences were statistically significant. While statistically significant differences were not present when comparing treatments for each combination, there were differences among rootstocks. Fruit weight from the OTC-treated trees with 'US-942', 'UFR-6', and 'US-897' rootstocks were significantly different from nontreated trees with 'Swingle', 'UFR-2', and 'UFR-4' rootstocks. Similar results were produced when data trees from the 2022–23 season were included ($P < 0.001$). In this analysis, all combinations produced a higher mean fruit weight when injected compared with their noninjected counterparts, except for 'US-942', which produced a marginally higher mean individual fruit weight when not injected. However, these differences were not statistically significant. For the 2023–24 season, OTC-treated trees produced a higher mean individual fruit weight compared with nontreated trees ($P < 0.001$).

There was no evidence for statistically significant differences in mean fruit weight between the two seasons ($P = 0.383$). When OTC-treated trees were removed from analysis, the 2022–23 season produced a higher mean fruit weight (171.55 g) compared with the 2023–24 season (161.37 g) ($P = 0.003$).

Fruit diameter. There were significant differences for mean fruit diameter when the 2022–23 season was analyzed separately (Table 1), as well as for the 2023–24 season when OTC-treated trees were included or removed from the analysis ($P < 0.001$, $P = 0.001$, and $P = 0.036$, respectively). There was evidence supporting statistically significant differences for mean fruit diameter among the different rootstock–'OLL-8' combinations evaluated in this study for nontreated trees across both seasons ($P < 0.001$). Fruit from trees with 'US-942' rootstock had the largest mean fruit diameter (70.7 mm), while trees with 'UFR-4' rootstock produced the smallest mean fruit diameter (65.5 mm).

Table 1. Rootstock selection impacts on external fruit qualities of the 'OLL-8' sweet orange scion for the 2022–23 season.

Rootstock	n	Fruit weight (g)	Fruit diameter (mm)	Fruit count	Total fruit weight (kg/tree)
UFR-2	12	163.9 c	66.4 c	251 a	41.0 a
UFR-4	12	158.6 c	65.5 c	258 a	40.9 a
UFR-6	12	180.2 b	68.6 b	164 b	29.0 ab
US-942	12	197.2 a	71.2 a	55 c	10.6 c
US-897	12	169.8 bc	67.0 bc	165 b	27.9 b
Swingle	12	159.6 c	65.4 c	198 ab	31.5 ab
P value		<0.001	<0.001	<0.001	<0.001

The external fruit quality variables evaluated were mean fruit weight (g), fruit diameter (mm), total fruit count, and total fruit weight per tree (kg/tree). Fruit was collected from a private grower in Haines City, FL, in Feb 2023, Mar 2023, and Apr 2023. The variable n indicates sample size. Means not sharing common letters indicate a significant difference between groups based on Tukey's honestly significant difference test ($P < 0.05$).

Table 2. OTC trunk injection and rootstock selection impacts on external fruit qualities of the 'OLL-8' sweet orange scion for the 2023–24 season.

Treatment × rootstock	*n*	Fruit weight (g)	Fruit diameter (mm)	Fruit count	Total fruit weight (kg/tree)
UFR-2	6	156.1 c	66.5 cd	322 a	49.7 a
OTC UFR-2	6	169.5 abc	68.2 abcd	305 a	50.7 a
UFR-4	6	153.5 c	65.6 d	344 a	52.8 a
OTC UFR-4	6	173.2 abc	68.7 abcd	305 a	52.3 a
UFR-6	6	168.3 abc	68.0 abcd	276 a	46.2 a
OTC UFR-6	6	186.1 a	70.5 ab	298 a	55.8 a
US-942	4	171.0 abc	69.1 abcd	79 a	13.8 a
OTC US-942	6	186.6 a	71.4 a	181 a	33.5 a
US-897	8	164.7 bc	67.6 bcd	291 a	47.7 a
OTC US-897	6	179.3 ab	69.7 abc	260 a	46.6 a
Swingle	6	156.8 c	66.1 cd	254 a	39.4 a
OTC Swingle	6	166.8 abc	67.5 bcd	275 a	45.9 a
P value		<0.001	<0.001	0.131	0.155
Treatments					
OTC-treated trees	36	176.9 a	69.3 a	271 a	47.5 a
Nontreated trees	36	161.4 b	67.0 b	273 a	43.5 a
P value		<0.001	<0.001	0.946	0.423

The external fruit quality variables evaluated were mean fruit weight (g), fruit diameter (mm), total fruit count, and total fruit weight per tree (kg/tree). Fruit was collected from a private grower in Haines City, FL, in Jan 2024 and Mar 2024. The variable *n* indicates sample size. Means not sharing common letters indicate a significant difference between groups based on Tukey's honestly significant difference test ($P < 0.05$).
OTC = Oxytetracycline.

When OTC-treated trees were included, similar results were produced, and fruit from trees with 'US-942' rootstock had a significantly larger mean fruit diameter than all other rootstock–'OLL-8' combinations evaluated ($P < 0.001$).

There was evidence for statistically significant differences in mean fruit diameter among treatments and rootstocks evaluated for the 2023–24 season (Table 2; $P < 0.001$). As with mean fruit weight, all combinations produced a marginally higher mean fruit diameter when treated with OTC, although none of these differences were statistically significant (Table 2). The highest mean fruit diameter was from OTC-treated trees with 'US-942' rootstock, which had a mean of 71.4 mm, and the lowest from nontreated trees with 'UFR-4' rootstock had a mean of 65.6 mm. The OTC-treated trees with 'US-942' and 'UFR-6' rootstock had a statistically higher mean fruit diameter than nontreated trees with 'UFR-2', 'Swingle', and 'UFR-4' rootstocks. When the 2022–23 data trees were included in the analysis, similar results were produced, in which statistically significant differences were found among treatments and rootstocks evaluated for mean fruit diameter ($P < 0.001$). However, in this analysis, OTC-treated trees with 'UFR-4' rootstock had a mean fruit diameter that was statistically greater than nontreated trees with 'UFR-4' rootstock. There was a significant positive and strong correlation between fruit diameter and fruit weight ($r = 0.969$, $P < 0.001$).

There was evidence for statistically significant differences among treatments for mean fruit diameter for the 2023–24 season ($P < 0.001$). Fruit from OTC-treated trees had a higher mean fruit diameter compared with nontreated trees. There was no evidence supporting statistically significant differences in mean fruit diameter between the two seasons, whether OTC-treated trees were included or removed ($P = 0.052$ and $P = 0.543$, respectively).

Juice quality testing

Titratable acidity. There was evidence for statistically significant differences in mean TA among rootstock–'OLL-8' combinations for the 2022–23 season (Table 3; $P < 0.001$). Mean TA ranged from 0.77% to 0.95%, which were represented by trees with 'US-942' rootstock and 'UFR-6' rootstock, respectively. Trees with 'US-942' rootstock produced juice with a mean TA significantly different from all other combinations evaluated. When both seasons of data were analyzed collectively, there was evidence for statistically significant differences when OTC-treated trees were removed ($P < 0.001$). As with the 2022–23 results, trees with 'US-942' rootstock had the lowest mean TA (0.77%), while the highest mean TA was from trees with 'Swingle' rootstock (0.94%). When OTC-treated trees were included in the analysis, the results were similar ($P < 0.001$). Interestingly, when OTC-treated trees were removed from the analysis for the 2023–24 season, there was no evidence supporting statistically significant differences in mean TA for the rootstock–'OLL-8' combinations evaluated ($P = 0.218$). However, when OTC-treated trees were included in this analysis for the 2023–24 season, there was evidence for statistically significant differences in mean TA among the rootstock–'OLL-8' combinations ($P < 0.001$).

There was evidence for significant differences in mean TA for treatments and combinations evaluated in this study for the 2023–24 season (Table 4; $P = 0.009$). Juice from OTC-treated trees with 'US-942' rootstock produced a mean TA of 0.74%, which was significantly lower than nontreated 'Swingle', which had the highest mean TA at 0.90%. This was the only statistically significant difference for TA in the 2023–24 season. There were also statistically significant differences in mean TA among treatments and combinations evaluated when the 2022–23 data were included in the analysis ($P < 0.001$). As with the 2023–24 season, all OTC-treated combinations had a marginally (not significantly different) lower mean TA compared with their noninjected counterparts; however, unlike with the 2023–24 season, OTC-treated trees with 'UFR-2' rootstock produced a significantly lower mean TA compared with nontreated 'UFR-2' trees. The OTC-treated trees with 'UFR-2' rootstock produced a 15% lower mean TA than nontreated trees with 'UFR-2' rootstock. Supplemental Table 1 reports the comparison of treatments and combinations for mean TA that includes data from the first and third harvest dates combined.

There was no evidence supporting significant differences in mean TA between OTC-treated and nontreated trees for the 2023–24 season regardless of rootstock ($P = 0.055$). The nontreated trees produced a mean TA of 0.86%, while the OTC-treated trees produced a mean of 0.81%. Interestingly, when comparing the mean TA of all data trees versus year, the 2022–23 season produced a significantly higher mean TA compared with the 2023–24 season, whether OTC-treated trees were included or removed from the analysis ($P < 0.001$ and $P = 0.005$, respectively).

Table 3. Rootstock selection impacts on fruit juice quality of the 'OLL-8' sweet orange scion for the 2022–23 season.

Rootstock	*n*	TA (%)	TSS (%)	Ratio (TSS:TA)	Pounds-solids per box	Juice color
UFR-2	12	0.95 a	8.63 a	9.23 a	4.49 a	37.55 a
UFR-4	11	0.92 a	8.67 a	9.53 a	4.47 a	37.55 a
UFR-6	12	0.95 a	8.95 a	9.43 a	4.68 a	37.69 a
US-942	8	0.77 b	7.56 b	9.85 a	3.63 b	37.78 a
US-897	11	0.90 a	8.79 a	9.82 a	4.73 a	37.93 a
Swingle	12	0.95 a	8.93 a	9.52 a	4.66 a	37.83 a
P value		<0.001	<0.001	0.131	<0.001	0.025

The juice quality variables evaluated were mean TA (%), TSS (%), ratio of TSS to TA, pounds-solids per box, and juice color. Fruit were collected from a private grower in Haines City, FL, USA in Mar 2023 and Apr 2023. The variable *n* indicates sample size. Means not sharing common letters indicate a significant difference between groups based on Tukey's honestly significant difference test ($P < 0.05$).
TA = titratable acidity; TSS = total soluble solids.

Table 4. OTC trunk injection and rootstock selection impacts on fruit juice quality of the 'OLL-8' sweet orange scion for the 2023–24 season.

Treatment × rootstock	TA (%)	TSS (%)	Ratio (TSS:TA)	Pounds-solids per box	Juice color
UFR-2	0.87 ab	8.67 ab	10.00 a	4.43 ab	38.13 a
OTC UFR-2	0.79 ab	9.00 ab	11.43 a	4.65 ab	38.13 a
UFR-4	0.88 ab	8.49 ab	9.74 a	4.31 ab	38.43 a
OTC UFR-4	0.80 ab	8.94 ab	11.35 a	4.57 ab	38.64 a
UFR-6	0.87 ab	8.89 ab	10.29 a	4.59 ab	38.63 a
OTC UFR-6	0.86 ab	9.66 a	11.21 a	5.07 a	38.81 a
US-942	0.77 ab	7.81 b	10.27 a	3.84 b	38.39 a
OTC US-942	0.74 b	8.29 b	11.44 a	3.94 b	38.76 a
US-897	0.85 ab	8.86 ab	10.52 a	4.64 ab	38.12 a
OTC US-897	0.81 ab	8.82 ab	10.95 a	4.68 ab	38.35 a
Swingle	0.90 a	8.97 ab	9.98 a	4.58 ab	38.21 a
OTC Swingle	0.89 ab	9.02 ab	10.18 a	4.72 ab	38.28 a
P value	0.009	0.007	0.02	0.006	0.175
Treatments					
OTC-treated trees	0.81 a	8.95 a	11.08 a	4.61 a	38.49 a
Non-OTC trees	0.86 a	8.63 a	10.15 b	4.41 a	38.31 a
P value	0.055	0.076	<0.001	0.150	0.150

The juice quality variables evaluated were mean TA (%), TSS (%), ratio of TSS to TA, pounds-solids per box, and juice color. Fruit were collected from a private grower in Haines City, FL, USA in Mar 2024. Means not sharing common letters indicate a significant difference between groups based on Tukey's honestly significant difference test ($P < 0.05$).

OTC = Oxytetracycline; TA = titratable acidity; TSS = total soluble solids.

Total soluble solids. There was evidence for significant differences for mean TSS among rootstock–'OLL-8' combinations for the 2022–23 season (Table 3; $P < 0.001$). Juice from trees with 'UFR-6' rootstock produced the highest mean TSS (8.95%). Trees with 'US-942' produced the lowest mean TSS (7.56%), which was different from all other rootstock–'OLL-8' combinations evaluated. Similar results were found for the 2023–24 season whether OTC-treated trees were included or removed from the analysis ($P = 0.002$ and $P = 0.029$, respectively). When OTC-treated trees were included in the analysis, juice from trees with 'UFR-6' rootstock had the highest mean TSS (9.27%) and when the OTC-treated trees were removed, trees on 'Swingle' rootstock produced the highest mean TSS (8.97%). There was evidence for statistically significant differences for mean TSS among rootstocks evaluated in this study for both seasons cumulative when OTC-treated trees were removed ($P < 0.001$). Juice from trees with 'US-942' rootstock produced the lowest mean TSS of 7.63%, which was different from all other combinations. The highest mean TSS was from trees with 'UFR-6' rootstock, with a mean TSS of 8.94% across both seasons. Similar results were found when including OTC-treated trees ($P < 0.001$). Juice from trees with 'UFR-6' rootstock were significantly different for mean TSS compared with juice from trees with 'UFR-4' and 'US-942' rootstocks.

There was evidence for statistically significant differences in mean TSS for rootstock–'OLL-8' combinations and treatments evaluated in this study for the 2023–24 season (Table 4; $P = 0.007$). The OTC-treated 'OLL-8' trees with 'UFR-6' rootstock produced juice with the highest mean TSS of 9.66%, which was statistically higher than juice from OTC-treated and nontreated 'OLL-8' trees with 'US-942' rootstock. Mean TSS values ranged from 7.63% to 9.66% for the rootstock–'OLL-8' combinations and treatments in this study. Most of the OTC-treated trees had a higher mean TSS compared with their nontreated counterparts, except for the trees with 'US-897' rootstock, in which the OTC-treated trees had a marginally lower mean, although these differences were not statistically significant. When treated with OTC, trees with 'UFR-6' rootstock produced a nearly 8% higher mean TSS than nontreated trees with 'UFR-6' rootstock. When data from the 2022–23 season were included in the analysis, the results were similar and indicated a statistically significant difference among rootstock–'OLL-8' combinations and treatments ($P < 0.001$). However, this analysis revealed more differences than the latter. The OTC-treated 'UFR-6' trees had the highest mean TSS, which was significantly different from nontreated trees with 'US-897', 'UFR-2', 'UFR-4', and 'US-942' rootstocks and OTC-treated trees with 'US-942' rootstock. Each of the rootstock–'OLL-8' combinations produced a marginally higher mean TSS when injected versus not injected; however, this difference was not statistically significant in any of the combinations. Supplemental Table 1 reports the comparisons of treatments and combinations for mean TSS that includes data combined from the first and third harvest dates.

There was no evidence for significant differences in mean TSS for the 2023–24 season between the two treatments ($P = 0.076$). The OTC-treated trees produced a mean TSS of 8.95% compared with nontreated trees with a mean of 8.63%. When the mean TSS of the 2022–23 and 2023–24 seasons were compared, there were no statistically significant differences whether OTC-treated trees were included or removed ($P = 0.197$ and $P = 0.887$, respectively).

Ratio. There was no evidence for significant differences in mean ratio among rootstock–'OLL-8' combinations for the 2022–23 season (Table 3; $P = 0.131$). Similar results were found for the 2023–24 season whether OTC-treated trees were included or removed ($P = 0.645$ and $P = 0.818$, respectively). When the 2022–23 and 2023–24 data were analyzed together, there was no evidence for significant differences in mean ratio among rootstock–'OLL-8' combinations whether OTC-treated trees were included or removed from the analysis ($P = 0.245$ and $P = 0.095$, respectively).

There was evidence for significant differences in mean ratio for the 2023–24 season among treatments and combinations evaluated; however, Tukey's honestly significant difference analysis was not able to validate differences among treatments (Table 4; $P = 0.020$). Mean ratios ranged from 9.74 and 11.44 for nontreated trees with 'UFR-4' rootstock and OTC-treated trees on 'US-942' rootstock, respectively. When the 2022–23 data were included in the analysis, there was evidence for significant differences among treatments and combinations for mean ratio ($P < 0.001$). All OTC-treated rootstock–'OLL-8' combinations outperformed all nontreated trees for mean ratio. Each of the OTC-treated trees except for OTC-treated trees with 'Swingle' rootstock had a mean ratio that was significantly higher than nontreated trees with 'Swingle', 'UFR-6', 'UFR-4', and 'UFR-2' rootstocks. The largest difference between treatments for mean ratio occurred for the trees with 'UFR-2' rootstock, which when treated with OTC increased the mean ratio by nearly 22%. The two other UFR rootstocks evaluated produced the second and third largest difference in ratio when treated with OTC; the mean ratio for trees with 'UFR-4' and 'UFR-6' rootstocks increased by 18.5% and 16.8%, respectively, when treated with OTC. Supplemental Table 1 reports the comparison of treatments and combinations for mean ratio that includes data combined from the first and third harvest dates.

There was evidence for statistically significant differences in mean ratio for OTC-treated and nontreated trees for the 2023–24 season ($P < 0.001$). Juice from OTC-treated trees had a mean ratio of 11.08, while nontreated trees had a mean ratio of 10.15.

There was evidence for statistically significant differences for mean ratio between both seasons of data collection whether OTC treatment was administered or not administered ($P < 0.001$). In both cases, the mean ratio was significantly higher during the 2023–24 season compared with the 2022–23 season.

Pounds-solids per box. There was evidence for statistically significant differences in mean pounds-solids per box among rootstock–'OLL-8' combinations for the 2022–23 season (Table 3; $P < 0.001$). Juice from trees with 'US-897' rootstock produced the highest mean pounds-solids per box, while trees with 'US-942' rootstock produced the lowest mean. Juice from trees with 'US-942' rootstock produced a mean that was statistically lower from all other rootstock–'OLL-8' combinations evaluated in this study. There was evidence for statistically significant differences in mean pounds-solids per box among rootstock–'OLL-8' combinations across both seasons when OTC-treated data trees were removed from analysis ($P < 0.001$). Juice from trees with 'US-897' rootstock produced significantly higher mean pounds-solids per box than trees with 'UFR-4' and 'US-942' rootstocks with means of 4.70, 4.44, and 3.69, respectively. When OTC-treated data trees were included in the analysis, trees on 'UFR-6' rootstock had the highest mean pounds-solids per box at 4.73, which was statistically different from the means of trees with 'UFR-4' and 'US-942' rootstocks. In contrast, when OTC-treated trees were removed from the analysis for the 2023–24 season, only trees with 'US-897', 'UFR-6', and 'Swingle' rootstocks had a statistically higher mean pounds-solids per box than that of trees with 'US-942' rootstock ($P = 0.007$). When OTC-treated trees were included in the analysis for the 2023–24 season, all combinations had a statistically higher mean pounds-solids per box than trees with 'US-942' rootstock, except for trees with 'UFR-4', which were not significantly different from each other.

There was evidence for statistically significant differences among treatments and combinations for pounds-solids per box for the 2023–24 season (Table 4; $P = 0.006$). Similar to ratio, the OTC-treated rootstock–'OLL-8' combinations produced a higher mean than their nontreated counterparts, although these differences were not significantly different. The OTC-treated trees with 'UFR-6' rootstock produced the highest mean pounds-solids per box at 5.07, while nontreated trees with 'US-942' rootstock produced the lowest mean at 3.84. The OTC-treated trees with 'UFR-6' rootstock had a mean pounds-solids per box that was significantly higher than both nontreated and OTC-treated trees with 'US-942' rootstocks. When data from the 2022–23 season was included in the analysis, OTC-treated trees with 'UFR-6' rootstock produced a significantly higher mean pounds-solids per box than nontreated trees with 'UFR-2', 'UFR-4', and 'US-942' rootstock and OTC-treated trees with 'US-942' rootstock.

There was no evidence for statistically significant differences in mean pounds-solids per box among treatments for the 2023–24 season ($P = 0.150$). The OTC-treated trees had a mean pounds-solids per box of 4.61, while nontreated trees had a mean of 4.41. There was no evidence for statistically significant differences in mean pounds-solids per box between the 2022–23 and 2023–24 seasons when OTC-treated trees were included ($P = 0.795$) or removed ($P = 0.453$).

Juice color

There was evidence for significant differences among rootstocks for mean juice color score for the 2022–23 (Table 3; $P = 0.025$) and 2023–24 seasons when OTC-treated trees were included ($P = 0.027$). However, when OTC-treated trees were removed from the analysis, there were no significant differences among rootstocks for the 2023–24 season ($P = 0.337$). Additionally, there was no evidence for statistically significant differences in mean juice color score among rootstocks evaluated across both seasons when OTC-treated trees were included or removed from analysis ($P = 0.235$ and $P = 0.251$, respectively).

For the 2023–24 season, there was no evidence for significant differences for mean juice color among treatments and combinations evaluated in this study (Table 4; $P = 0.175$). However, when data from 2022–23 was included in the analysis, there was evidence for statistically significant differences in mean juice color ($P < 0.001$). The highest mean juice color score was recorded from the OTC-treated trees with 'UFR-6' rootstock (38.81), which was significantly higher from nontreated trees with 'Swingle', 'UFR-6', 'UFR-4', and 'UFR-2' rootstocks. All nontreated rootstock–'OLL-8' combinations had a marginally lower mean juice color score compared with all OTC-treated combinations, although this difference was not significant. The mean juice color score was significantly higher in OTC-treated trees with 'UFR-6' and 'UFR-4' as rootstocks compared with their nontreated counterparts, respectively. Supplemental Table 1 contains the comparisons of treatments and combinations for mean juice color that includes combined data from the first and third harvest dates.

There was no evidence for statistically significant differences in mean juice color score among the OTC-treated and nontreated trees ($P = 0.150$). Overall, mean juice color score was significantly higher for the 2023–24 season compared with the 2022–23 season when OTC-treated trees were included in the analysis ($P < 0.001$) or removed ($P < 0.001$).

Fruit yields

Total fruit count yield per tree. For the 2022–23 season, the mean fruit count of 'OLL-8' was significantly lower for those with 'US-897', 'UFR-6', and 'US-942' rootstocks compared with the means for trees with 'UFR-4' and 'UFR-2' rootstocks (Table 1; $P < 0.001$). When all nontreated trees across both seasons were analyzed, there was evidence for significant differences for mean fruit count among the different rootstocks evaluated in this study ($P < 0.001$). The lowest mean fruit count was for trees with 'US-942' rootstock with a mean of 61 fruits/tree, which was significantly lower than the mean fruit counts from all other rootstock–'OLL-8' combinations. The trees with 'UFR-4' rootstock had the highest mean fruit count with 286 fruits/tree. Similar results were found when including the OTC-treated trees where the mean for trees with 'US-942' was significantly lower than all other combinations ($P < 0.001$). For the 2023–24 season, when OTC-treated trees were included in the analysis, the fruit count for trees with 'UFR-4' and 'UFR-2' rootstocks produced a significantly higher mean than those with 'US-942' rootstock.

Despite the range of mean fruit count per tree ranging from 79 to 344 for the 2023–24 season, there was no evidence for statistically significant differences among treatments and combinations for number of fruits/tree (Table 2; $P = 0.131$). The highest mean fruit count was recorded from nontreated trees with 'UFR-4' rootstock, while the lowest was from nontreated trees with 'US-942' rootstock. When data from the 2022–23 season was included in the analysis, there was evidence for significant differences among treatments and combinations for mean fruit count per tree ($P < 0.001$). In this analysis, nontreated trees with 'US-942' had a significantly lower mean fruit count than all other treatments and combinations except for OTC-treated trees with 'US-942' rootstock. The three 'UFR' rootstocks evaluated in this study had the highest mean fruit counts, although their mean fruit counts were only statistically higher than the mean fruit count of nontreated trees with 'US-942' rootstock.

In the 2023–24 season, there was no evidence supporting a significant difference in mean fruit counts between the two treatments. The nontreated trees produced a mean fruit count of 273 fruits/tree, while the OTC-treated trees produced a mean of 271 fruits/tree. For each of the two seasons evaluated, trees with 'UFR-4' and 'UFR-2' rootstocks produced the highest fruit count among all combinations, although these differences were not significant.

Total fruit weight yield per tree. There was evidence for statistically significant differences in mean total fruit weight yield per tree among rootstock–'OLL-8' combinations for the 2022–23 season (Table 1; $P < 0.001$). Trees with 'UFR-2' and 'UFR-4' rootstocks produced a significantly higher mean total fruit weight yield per tree compared with trees with 'US-897' and 'US-942' rootstocks. Similarly, when the rootstock–'OLL-8' combinations were evaluated across both seasons, there was evidence for significant differences in mean total fruit weight yield per tree ($P < 0.001$). The trees with 'US-942' rootstock produced the lowest mean fruit weight yield of 11.4 kg/tree, which was significantly lower than means from all other combinations (Fig. 1). For the 2023–24 season, when OTC-treated trees were removed from the analysis, there was no evidence for statistically significant differences among rootstock–scion combinations evaluated ($P = 0.152$). However, when the OTC-treated trees were included, there was evidence for statistically significant differences among the rootstock–'OLL-8' combinations evaluated ($P = 0.026$). In this analysis, trees with 'UFR-4' and 'UFR-6' rootstocks produced a significantly higher mean total fruit weight yield than trees with 'US-942'.

There was evidence for significant differences among treatments and rootstocks for mean fruit weight yield when 2022–23 and 2023–24 data were included in the analysis ($P < 0.001$). The nontreated trees with 'US-942' rootstock produced a significantly lower mean total fruit weight than all other treatments and rootstocks except for OTC-treated

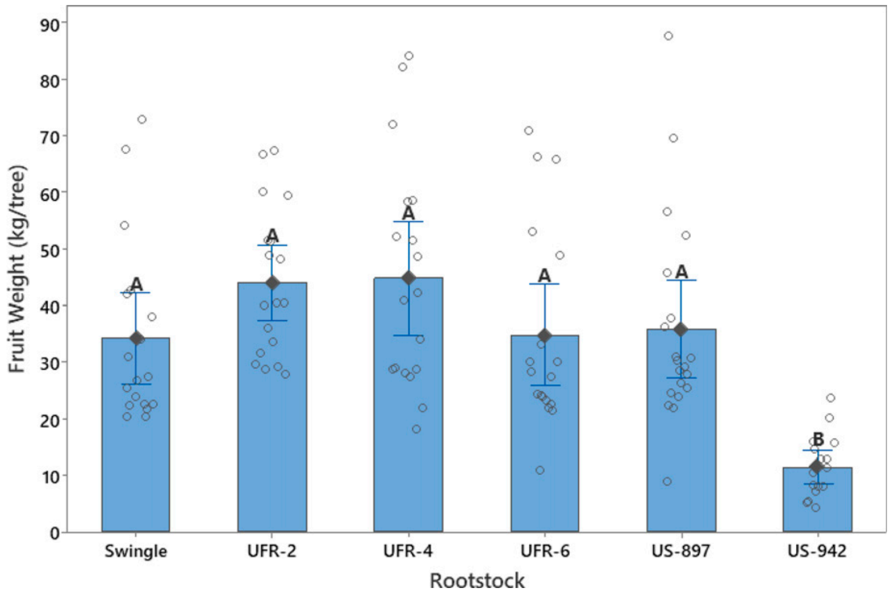

Fig. 1. Individual value plot displaying mean total fruit weight per tree (in kg) for each rootstock across both seasons of data collection. Each circle represents the total fruit weight produced by an individual tree. Each solid diamond represents the mean total fruit weight for a rootstock–'OLL-8' combination. Each interval bar displays a 95% confidence interval around the mean of a rootstock evaluated. Means not sharing common letters indicate a significant difference ($P < 0.05$) between groups based on Tukey's honestly significant difference test.

trees with 'US-942'. The top five mean total fruit weights all belonged to OTC-treated rootstocks and are in order from greatest to lowest as follows: 'UFR-6', 'UFR-4', 'UFR-2', 'US-897', and 'Swingle'. The OTC-treated trees with 'UFR-6' rootstock produced the highest mean total fruit weight yield per tree at 55.8 kg/tree, while the lowest mean fruit weight yield was from nontreated trees with 'US-942', which had a value of 11.4 kg/tree. The mean total fruit weight yield for treated trees with 'US-942' rootstock was 33.5 kg/tree, which is nearly 3-fold higher than the mean for noninjected trees with 'US-942' rootstock. It is important to note, however, that when the 2022–23 data were removed from the analysis, there was no evidence for significant differences among the treatments and combinations evaluated in this study (Table 2; $P = 0.155$).

There was no evidence for statistically significant differences between treatments for mean total fruit weight yield ($P = 0.423$). The OTC-treated trees had a mean total fruit weight of 47.5 kg/tree, while the nontreated trees had a mean of 43.5 kg/tree for the 2023–24 season.

Overall, there was evidence for significant differences in mean total fruit weight yield between the two seasons when OTC-treated trees were removed from the analysis ($P < 0.001$). The mean fruit weight yield was 30.2 kg/tree for the 2022–23 season and 45.5 kg/tree for the 2023–24 season. Even when OTC-treated trees were included, there was evidence for statistically significant differences between the two seasons for mean total fruit weight yield per tree ($P < 0.001$).

Premature fruit drop. There was no evidence for statistically significant differences in mean fruit drop for Mar 2024 data collection among rootstocks and treatments evaluated in the study ($P = 0.144$). The mean fruit drops ranged from 26 to 43 pieces of fruit for OTC-treated trees with 'US-942' and nontreated trees with 'UFR-6', respectively. However, there was evidence for statistically significant differences in mean fruit drop among treatments evaluated ($P = 0.006$). The nontreated trees produced a mean fruit drop of 39 fruits/tree, while the OTC-treated trees produced a mean of 32 fruits/tree. Fruit drop data were also collected in January, and the results were similar, with nontreated trees having a higher mean fruit drop compared with the OTC-treated trees ($P = 0.003$).

Pounds-solids per hectare. There was evidence for significant differences in mean pounds-solids per hectare among the rootstock–'OLL-8' combinations for the 2022–23 season ($P < 0.001$). In contrast, there were no differences among rootstock–'OLL-8' combinations for mean pounds-solids per hectare for the 2023–24 season, when OTC-treated trees were removed ($P = 0.152$); however, when the OTC-treated trees were included, there was evidence for significant differences in mean pounds-solids per hectare among rootstocks evaluated ($P = 0.022$). When all nontreated trees across both seasons were analyzed for differences in mean pounds-solids per hectare, there was evidence for significant differences ($P < 0.001$). The nontreated 'US-942' trees had a significantly lower mean pounds-solids per hectare compared with all other rootstocks evaluated (Fig. 2). Similarly, when OTC-treated trees were included, there was evidence for statistically significant differences in mean pounds-solids per hectare among the rootstocks evaluated.

There was no evidence for statistically significant differences in mean pounds-solids per box among the treatments and rootstock–'OLL-8' combinations evaluated in the 2023–24 season ($P = 0.068$). When all trees sampled in this study were analyzed together, there was evidence for significant differences in mean pounds-solids per hectare ($P < 0.001$).

For the 2023–24 season, there was no evidence that indicated a statistically significant difference in mean pounds-solids per hectare between the two treatments, despite the fact that treated trees had a mean of 3378 pounds-solids per hectare, while the nontreated trees had a mean of 2841 ($P = 0.132$). There was evidence for a significant difference in mean pounds-solids per hectare among the two seasons of data collection, whether OTC-treated trees were included ($P < 0.001$) or removed ($P = 0.015$). The 2022–23 season resulted in a mean pounds-solids per hectare of 2198, and the 2023–24 season resulted in a mean of 2841 when OTC-treated trees were removed and a mean of 3110 when included.

Discussion

The results in this study demonstrate that OTC trunk injections can affect important fruit and juice quality characteristics, as well as yields of the 'OLL-8' sweet orange scion. Additionally, results demonstrate that rootstock selection for the 'OLL-8' scion can affect fruit and juice quality, as well as yield, which is similar to 'Valencia' sweet orange in Florida, of which fruit and juice quality is significantly affected by rootstock selection (Bowman et al. 2016b).

Rootstock selection can significantly affect fruit and juice quality. Rootstock selection can significantly affect important fruit juice quality characteristics, disease severity, and yields in conjunction with different sweet orange varieties (Continella et al. 2018; Treeby et al. 2007). The results of this experiment provide evidence that rootstock selection can affect fruit juice quality and yield of the 'OLL-8' sweet orange scion. A recent study conducted in Brazil evaluated the effects of 20 different rootstock selections in conjunction with the 'Valencia' sweet orange scion and found significant differences for TSS, TA and yield, characteristics (Domingues et al. 2021). In the present study, trees on 'US-942' rootstocks had significantly lower yields compared with the other rootstocks evaluated. In contrast, many studies evaluating rootstock effects have found that trees on 'US-942' typically have higher production than indicated by the results in this study. Bowman et al. (2016b) found that at 5 to 6 years of age, 'Valencia' trees on 'US-942' rootstocks had a significantly higher yield (kg/tree) than 'Valencia' on 'US-897' for 2013 and 2014. Bowman et al. (2016b) also found that 'Valencia' on 'US-942' had significantly higher yields for 2012 and 2013 compared with 'Valencia' on 'Swingle'. These results disagree with those found in the current study. This could be due to differences in scion; the previous study included 'Valencia',

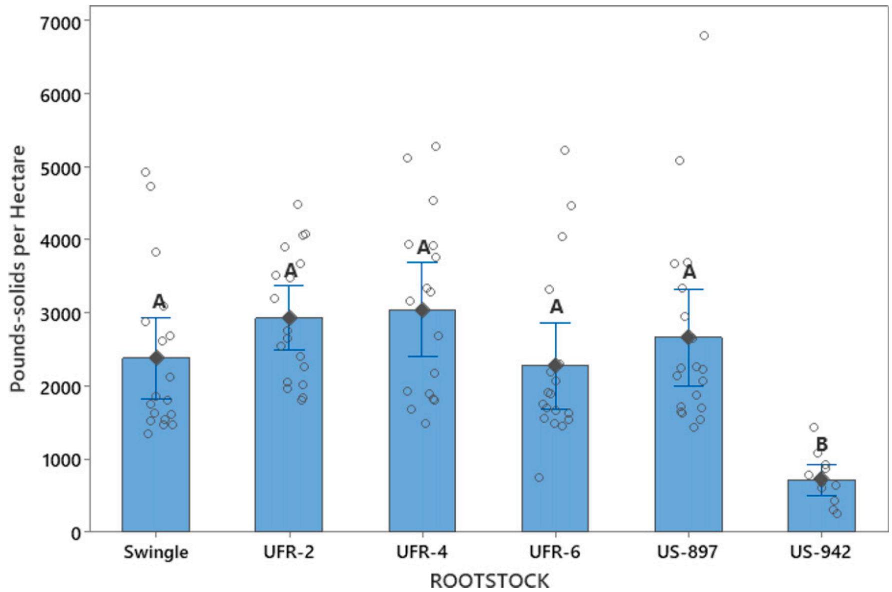

Fig. 2. Individual value plot displaying pounds-solids per hectare across the 2022–23 and 2023–24 seasons for each rootstock–'OLL-8' combination evaluated in this study. Each circle represents an individual data point, while each solid diamond represents the overall mean for a rootstock–'OLL-8' combination. Each interval bar displays a 95% confidence interval around the mean of a rootstock. Means not sharing common letters indicate a significant difference ($P < 0.05$) between groups based on Tukey's honestly significant difference test.

while the current study evaluates the 'OLL-8' sweet orange scion. In the current study, trees on 'US-942' rootstock had an earlier planting date; however, these trees were close enough in age to those on 'US-897' and the trees used in the Bowman et al. (2016b) study. Bowman et al. (2016b) reported a mean yield of 57 kg/tree for 'Valencia' on 'US-942', whereas 'Valencia' trees on 'US-897' rootstock had a mean yield of 34 kg/tree. These results differ from those found in the current study, in which 'OLL-8' on 'US-942' had a mean yield of 12.2 kg/tree, and 'OLL-8' on 'US-897' produced a mean yield of 37.8 kg/tree. In another field study conducted by Bowman et al. (2016a), 'Minneola' grafted on 'US-942' and 'US-897' produced the highest yields for five separate seasons and had significantly higher cumulative yields across the five seasons compared with trees on 'Swingle' and other USDA-released rootstocks.

For each of the seasons evaluated, trees on 'US-942' exhibited the lowest TSS and were significantly different from the other rootstocks evaluated. The exception to this was in the 2023–24 season, in which trees on 'US-942' and 'UFR-4' rootstocks were not significantly different. The mean TSS across both seasons for 'US-942' trees was 7.64%, which is lower than reported by other studies. Bowman et al. (2016b) recorded a mean TSS of 9.73% for 5- to 6-year-old trees on 'US-942' with 'Valencia' scions. Once again, the age of the trees reported by Bowman et al. (2016b) are similar to those in this study, alluding to potential rootstock–scion effects on fruit and juice quality of the 'OLL-8' sweet orange scion. In the same study, trees on 'US-897' and 'Swingle' had mean TSS values of 9.81% and 9.19%, respectively. These results are closer to those found in this study, in which 'US-897' and 'Swingle' trees had mean TSS values of 8.84% and 8.94%, respectively. In this study, trees on 'UFR-6' had the highest mean TSS across both seasons compared with all other rootstocks evaluated in this study. Kunwar et al. (2023) reported similar results, with 'Valencia' on 'UFR-6' having a mean TSS of 9.53% across three seasons and having a higher mean TSS than 'Valencia' on 'UFR-2', 'UFR-4', 'US-897', and 'Swingle', although these differences were not statistically significant.

The TA values reported in the current study may be relatively high due to the premature harvest dates and variable climate conditions, including the hurricane event that occurred in 2023. Kunwar et al. (2023) reported the following TA values for 'Valencia' on 'UFR-2', 'UFR-4', 'UFR-6', 'US-897' and 'Swingle', respectively: 0.57%, 0.61%, 0.64%, 0.63%, and 0.64%. In the current study, each of the latter rootstocks produced a mean TA over 0.90% for the 2022–23 season, and a mean TA over 0.84% for the 2023–24 season, when the OTC-treated trees were removed from the analysis. In turn, the ratio of the juice samples obtained in the study may have also been relatively low compared with those found in the study by Kunwar et al. (2023) due to the reasons mentioned above. Interestingly, the color scores in this study across both seasons for 'OLL-8' on 'UFR-2', 'UFR-4', 'UFR-6', 'US-897', 'Swingle', and 'US-942', ranged from 37.66 to 37.98, which were higher than those reported by Kunwar et al. (2023) for 'Valencia' on 'UFR-2', 'UFR-4', 'UFR-6', 'US-897', and 'Swingle', which ranged from 36.1 to 36.4. In contrast, Bowman et al. (2016b), reported mean juice color scores for 'Valencia' on 'US-942', 'US-897', and 'Swingle' ranging from 38.2 to 38.6, which were closer to the scores reported in this study. Based on this information, the 'OLL-8' sweet orange scion produces juice color scores similar to those associated with 'Valencia' values.

Pounds-solids per hectare is an important metric to disseminate to growers that allows for a more informed planting decision as it relates to variety selection and profitability. In this study, calculations for pounds-solids per hectare were performed only on trees that were fully harvested. This allows for a comprehensive overview of production values, compared with sampling only a portion of the tree, in which human error may result in relatively more nonsymptomatic fruits being sampled and may not represent the mean produced by the entire crop load for that tree. In this study, pounds-solids per hectare are reported; however, they can be easily converted to pounds-solids per acre, which allows for easier comparison with studies using pounds-solids per acre. Singerman et al. (2021) analyzed pounds-solids per acre of 'Valencia' on different rootstocks across multiple sites in Florida. The values produced at the Babson Park site in the latter study are similar to those produced in the current study. Trees on 'UFR-2' produced a mean pounds-solids per acre of 813.23 for the 2019–20 season, whereas in this study, 'OLL-8' on 'UFR-2' produced a mean pounds-solids per acre of 1308 for the 2023–24 season. Singerman et al. (2021) reported that 'Valencia' on 'UFR-4' produced a mean pounds-solids per acre of 1144.65, which is close to the mean in this study for 'OLL-8' on 'UFR-4', which was 1342. The largest difference in the current study and the values reported by Singerman et al. (2021) was for trees on 'US-942' rootstock. The current study produced a mean pounds-solid per acre of 319.4 for the 2023–24 season, while Singerman et al. (2021) reported a mean of 1279.19. This large difference could potentially be attributed to the different rootstock influence on different scions ('Valencia' versus 'OLL-8') and/or increase in HLB severity. Another possibility is that there may be unknown differences between seed-propagated and tissue culture–propagated lines of 'US-942' rootstock.

OTC trunk injections can improve fruit and juice quality. HLB has devastated the Florida citrus industry since its first identification in 2005, including a decrease in acreage dedicated to citrus, as well as significantly decreasing quality characteristics of both fruit and juice. Many avenues have been explored for potential solutions to this increasing problem, including developing resistant varieties (Albrecht and Bowman 2012; Domingues et al. 2021), enhanced nutrient programs (Kwakye and Kadyampakeni 2022), and OTC trunk injections (Hu et al. 2018). The results of this experiment provide strong evidence that OTC trunk injections can significantly affect important fruit and juice quality as well as yield (fruit drop) characteristics in sweet orange, although rootstock is also a factor in outcome. It is important to note that fruit from OTC-injected trees during the 2023–24 season were harvested ~250 d (first harvest), 290 d (second harvest), and 310 d (final harvest) from the injection date in May 2023. Hu and Wang (2016) found that fruit from OTC-injected trees had a detectable OTC concentration of 202 μg/kg 270 d postinjection, which was 42% below the level established by the US EPA.

Similar to the results reported in this study, Hu and Wang (2016) reported that OTC-injected sweet orange trees exhibited a lower mean TA compared with water-injected and noninjected trees. While in both cases the difference was not statistically significant, this decrease is promising and might suggest that multiple injections over multiple seasons may be necessary for statistically significant changes. Overall, the mean TAs presented in this study were similar to those reported for ‘Valencia’ by the USDA NASS (2024). Juice TSS was also affected by OTC treatment in this study. Two of the rootstocks evaluated (‘US-942’ and ‘UFR-6’) exhibited a ~7% increase in TSS when injected versus not injected. A similar difference in mean TSS was reported by Archer et al. (2023) for ‘Valencia’, with OTC-injected trees having a mean TSS 8.5% higher than noninjected ‘Valencia’ trees. It is important to consider that although the trees were mature and of similar size, those on ‘US-942’ were 5 to 6 years old (in the field), while the other data trees were slightly older. When taken together, ‘US-942’ may not be the best choice of rootstock for ‘OLL-8’.

Perhaps the most significant impact that OTC trunk injections had on fruit and juice quality was exhibited in the analysis of the ratio. The difference in mean ratio between noninjected and injected trees was greatest in the UFR rootstocks. The UFR rootstocks used in this experiment, ‘UFR-2’, ‘UFR-4’, and ‘UFR-6’ exhibited a 22%, 18.5%, and 16.75% increase in mean ratio when injected, respectively. Other studies have also reported a significantly higher mean ratio in OTC-treated trees (Castellano-Hinojosa et al. 2024) and OTC- and streptomycin-treated trees (Hu et al. 2018), while others have reported no statistically significant differences (Archer et al. 2023; Hu and Wang 2016) among an array of sweet orange varieties.

Interestingly, OTC treatment had a significant impact on mean juice color scores for the treatments and combinations evaluated in this experiment. All OTC treatments produced a higher mean color score compared with their noninjected counterparts. However, it is important to mention that “Grade A” orange juice, as characterized by the US Department of Agriculture (2021), has a color score of 36 to 40; all mean juice color scores reported in this study met this requirement regardless of rootstock and treatment. However, other standards must be met to be classified as “Grade A” orange juice, including minimum TSS and ratio requirements, which a majority of samples did not meet in this study for specifications that existed during the two seasons evaluated for this experiment. The values for fruit and juice quality were likely relatively low for the ‘OLL-8’ scion due to HLB and the earlier than normal harvest time, as requested by the grower.

Conclusions

This study demonstrates that OTC trunk injection improved fruit and fruit juice quality of the ‘OLL-8’ sweet orange scion after one injection. Fruit from the OTC-treated trees had greater mean ratios, diameters, and weights, and the OTC treatment also decreased premature fruit drop, regardless of rootstock. There was strong evidence that, in the HLB environment, rootstock selection has an impact on fruit and fruit juice quality, as well as whole-tree yield. Overall, trees grafted onto ‘US-942’ produced larger and heavier fruit, although the trees exhibited the lowest crop load of the rootstocks evaluated. Interestingly, these trees had the lowest mean TA, TSS, and lowest pounds-solids per box of the rootstocks evaluated. The three UFR rootstocks in this study performed similarly if not better than the three commercial standards for many of the variables evaluated. The results of this experiment indicate that OTC trunk injection and rootstock selection can significantly improve the performance of HLB-affected trees, and there was no evidence of an interaction between rootstock and OTC treatment. Further data collection across multiple seasons and locations is necessary to confirm rootstock performance and the positive benefits of OTC trunk injection as well as the potential effects of long-term applications.

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