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Nutritional Quality of Field-grown Tomato Fruit as Affected by Grafting with Interspecific Hybrid Rootstocks

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***Abstract*. In this study, the effects of grafting with interspecific hybrid rootstocks on field-grown tomato fruit quality were evaluated over a 2-year period. Fruit quality attributes from determinate 'Florida 47' tomato plants grafted onto either 'Beaufort' or 'Multifort' rootstocks were compared with those from non- and self-grafted controls. Grafted plants had higher fruit yields than non- and self-grafted plants, and increased production of marketable fruit by ≈41%. The increased yield was accompanied by few major differences in nutritional quality attributes measured for these fruit. Although grafting with the interspecific rootstocks led to consistently small, but significant increases of fruit moisture (≈0.6%), flavor attributes such as total titratable acidity (TTA) and the ratio of soluble solids content (SSC) to TTA were not significantly altered. Among the antioxidants evaluated, ascorbic acid concentration was reduced by 22% in fruit from grafted plants, but significant effects were not evident for either total phenolics or antioxidant capacity as assayed by oxygen radical absorbance capacity (ORAC). Levels of carotenoids (lycopene, β-carotene, and lutein) were similar in fruit from grafted plants with hybrid rootstocks compared with non- and self-grafted controls. Overall, the seasonal differences outweighed the grafting effects on fruit quality attributes. This study showed that grafting with interspecific hybrid rootstocks could be an effective horticultural technique for enhancing fruit yield of tomato plants. Despite the modest reduction in ascorbic acid content associated with the use of these rootstocks, grafting did not cause major negative impacts on fruit composition or nutritional quality of fresh-market tomatoes.**

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop widely grown and consumed (fresh and processed forms) throughout the world. Tomato fruit are rich sources of nutritional components with antioxidant activity such as vitamin C, phenolics, and carotenoids, particularly lycopene (Burri et al., 2009; Jones et al., 2003). Consumption of foods containing these antioxidant compounds has been suggested to provide some level of protection against harmful free radicals and thus reduce risk of cancer and cardiovascular diseases (Giovannucci, 2002).

Tomato is susceptible to more than 200 diseases caused by pathogenic fungi, bacteria, viruses, or nematodes (Lukyanenko, 1991). In diverse production regions around the world, a wide range of biotic and abiotic stresses hinder the growth and development of this crop, and may cause significant economic losses. In addition to breeding disease-resistant cultivars, integrated pest management practices such as grafting have been successfully implemented to control several soil-borne diseases and root-knot nematodes in tomato production (Louws et al., 2010). These approaches have been especially effective under intensive cultivation (Lee et al., 2010; Rivard et al., 2010). Disease-resistant and interspecific tomato hybrid rootstocks have also been developed (King et al., 2010). Moreover, vegetable grafting has been used successfully to minimize the deleterious effects of a wide range of abiotic stresses related to salinity (Colla et al., 2010), nutrient and heavy metals (Savvas et al., 2010), water, and temperature (Schwarz et al., 2010).

Overall, the influence of rootstocks on the quality of fruits from grafted vegetable crops such as tomato and melon remains poorly understood, especially considering the continuous development of new rootstocks with disease resistance packages and vigorous growth characteristics and the various possible rootstock–scion combinations. Further research is clearly needed to understand the complex interactions potentially involved. For example, a study of melon plants grafted onto different *Cucurbita* spp. rootstocks showed a consistent decrease in taste and texture for some, but not all, combinations (Traka-Mavrona et al., 2000), whereas another study on sweet bell pepper showed no difference between grafted and nongrafted plants in the nutritional quality parameters evaluated (Colla et al., 2008). Furthermore, findings of improved nutritional quality of tomato fruit by grafting have also been reported (Flores et al., 2010). To ensure successful marketing and profitability for grafted tomatoes, the cost of grafting must be offset by enhanced marketable fruit yields with equal or better quality. In previous studies of fruit quality of grafted tomatoes, results have varied depending on the scion–rootstock combinations. In some instances, there were no significant differences in TTA or SSC (Khah et al., 2006). In others, grafting increased the levels of lycopene, β-carotene, vitamin C, and antioxidant activity in tomato fruit (Dorais et al., 2008; Fernandez-Garcia et al., 2004). These findings that sometimes appear contradictory are largely due to the complexity of the biochemical processes that determine the synthesis of these various compounds that define the tomato fruit sensory and nutritional quality. These phytochemicals can be affected by various biotic and abiotic factors. Among these are cultivar, production practices, maturity at harvest, and the environmental conditions before and after harvest (Dorais et al., 2008; Foolad, 2007; Simonne et al., 2011). An adequate supply of potassium, for example, enhances the titratable acidity of tomato fruit (Adams, 2002). Also, abundant nitrogen may decrease fruit quality by reducing the sugar (Parisi et al., 2004) and vitamin C contents (Simonne et al., 2007).

Similar effects may occur in fruit of grafted tomatoes, since rootstocks can influence the plant nutrient status, water relations, and other physiological processes. In addition to these changes, rootstocks could alter fruit quality through direct transfer of metabolites via xylem from root to fruit (Lee, 1994; Rouphael et al., 2010). Scion–rootstock interactions may also be involved, since most of the currently available, interspecific, hybrid rootstocks were developed directly from wild species. These rootstocks tend to increase plant vegetative growth and fruit yields (Di Gioia et al., 2010; Leonardi and Giuffrida, 2006) to such an extent that potential for an accompanying modification of fruit quality needs to be carefully examined. In fact, a negative correlation between sugars and fruit yield has been reported, which was suggested to be associated with the high ratio of fruit/leaf tissue in high yielding plants limiting the supply of

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carbohydrates to fruit (Georgelis et al., 2004). With new rootstocks becoming available for grafted tomato production, comparative studies become still more important for better understanding the degree and mechanisms of possible rootstock influence on fruit quality. It is also noteworthy that previous studies on fruit quality of grafted vegetables were oftentimes focused on protected production systems rather than the open-field systems used in this study. With respect to grafted tomato production in the United States, a number of studies have clearly demonstrated the great potential of using grafted plants for controlling several soil-borne pathogens and increasing plant vigor and fruit yield (Barrett et al., 2012a; King et al., 2008; Louws et al., 2010; McAvoy et al., 2012; Rivard et al., 2012); however, research is lacking as to whether the plant growth modifications as a result of grafting with vigorous rootstocks could also lead to marked changes in fruit quality properties especially with field-grown, large fruit size tomato cultivars. Therefore, it is hypothesized that grafting onto interspecific hybrid rootstocks does not adversely affect the major intrinsic quality measurements of fresh-market tomato fruit.

The main objective of this study was to test the extent of interspecific hybrid rootstock effects on quality of field-grown tomato fruit by determining changes in fruit pH, SSC, TTA, antioxidant activity, and levels of vitamin C (ascorbic acid), carotenoids, and total phenolics.

Materials and Methods

Experimental design and fruit sampling. Tomato fruit were sampled from field experiments carried out at the University of Florida Suwannee Valley Agricultural Extension Center in Live Oak, FL (30.31° N, 82.90° W) during the spring seasons of 2010 and 2011. Four treatments of the determinate tomato cultivar 'Florida 47' (Seminis Vegetable Seeds, Inc., St. Louis, MO) were compared including 1) nongrafted 'Florida 47' (FL), 2) self-grafted 'Florida 47' (FL/FL), 3) 'Florida 47' grafted onto 'Beaufort' (FL/BE), and 4) 'Florida 47'grafted onto 'Multifort' (FL/MU). Both 'Beaufort' and 'Multifort' (De Ruiter Seeds Inc., Bergschenhoek, The Netherlands) are commercially available interspecific tomato hybrid rootstocks (*S. lycopersicum* × *Solanum habrochaites* S. Knapp & D.M. Spooner) with greater resistance or tolerance to soil-borne pathogens. The self-grafted scion plants (FL/FL) were included as another control to test whether the grafting process per se has any impact on the fruit quality attributes evaluated as opposed to the effects of rootstocks and their interactions with the tomato scion. Plants were grown in plastic mulched beds with a full regime of drip irrigation and total nitrogen applied at 224 kg·ha^{-1} (Djidonou et al., 2013). The experiment was arranged in a randomized complete block design with four replications (blocks), and 12 plants per replication. Fruit at the mature green stage of ripeness or more advanced ripening stages were harvested at 80 (June 16) and 75 (June 14) days after transplanting (DAT) in 2010 and 2011, respectively. Using the U.S. Standard Grades for fresh-market tomatoes (USDA, 1997), fruit were then graded as extra-large, large, medium, and culls (small fruit and defective fruit) for yield determination. Fruit in the extra-large, large, and medium grades were considered marketable. After counting and weighing fruit in each grade size, fruit graded as large (63.5–70.6 mm fruit diameter) were kept separately (per treatment per replication) and were used to evaluate the fruit quality attributes. More specifically, at each harvest, 8 to 10 of these fruit (at breaker stage, i.e., fruit showing a clear break in color from green to tannish yellow) of similar size and color development were randomly selected from each treatment per replication and stored at 20 °C until fully red.

Sample preparation. When fruit reached full ripeness, they were sliced and homogenized in a Waring blender for 1 min. Freshly homogenized samples were then stored at –30 °C and used for measurements of pH, TTA, and SSC. Additional homogenized samples were stored at –80 °C and later used to quantify levels of carotenoids (lycopene, β-carotene, and lutein), phenolics (hydrophilic and lipophilic), and antioxidant activity. For determination of ascorbic acid content, 2 g of the freshly homogenized fruit tissues were preserved in 20 mL acid mixture (6% metaphosphoric acid with 2 N glacial acetic acid) and stored at –30 °C.

Determination of moisture content, pH, SSC, and TTA. Moisture content was measured using AOAC method 920.151 (AOAC, 2000). Briefly, 2 g samples of the freshly homogenized fruit tissues were weighed into aluminum pans and dried using a vacuum pressure oven at 60 °C and 101.6 kPa for 48 h.

For TTA determinations, frozen homogenized fruit tissues (–30 °C) were thawed, then centrifuged at 4 °C and 17,600 g_n for 20 min. The supernatant was filtered through cheesecloth before analysis. The TTA was determined by titrating 6.0 g of juice plus 50 mL of deionized water with 0.1 N sodium hydroxide solution until pH 8.2. An automatic titrimeter (Titrino 719 S model; Metrohm, Herisau, Switzerland) was used, and the TTA was expressed as percent of citric acid. The pH of the diluted juice was determined automatically by the pH electrode of the titrimeter. The SSC of the supernatant was measured with a digital refractometer (model ABBE Mark II; Reichert Technologies, Depew, NY).

Determination of ascorbic acid content. The ascorbic acid (vitamin C) content of tomato fruit was measured spectrophotometrically based on AOAC method 967.21 (AOAC, 2000). Briefly, 2 g of the freshly homogenized fruit tissues in 20 mL of 6% metaphosphoric acid in 2 N glacial acetic acid mixture was centrifuged at 4 °C and 17,600 g_n for 20 min. The supernatant was filtered through Whatman #4 filter paper before analysis. Triplicate, 1 mL aliquots of clear supernatant from each sample were pipetted into test tubes, and vortexed after addition of 0.05 mL of 0.2% 2,6-dichlorophenolindophenol. All reaction mixtures were held at room temperature for 1 h, then mixed well with 1 mL of 2% thiourea in 5% metaphosphoric acid, and 0.5 mL of 2% dinitrophenylhydrazine in 9 N H_2SO_4. Reaction mixtures were subsequently incubated in a water bath at 60 °C for 3 h followed by slow addition of 2.5 mL ice-cold 90% (v/v) H_2SO_4. Thereafter, the absorbance of 250 µL samples was measured at 540 nm using a microplate spectrophotometer (model Power Wave X52; BioTek Instruments, Inc., Winooski, VT). The vitamin C content of samples was calculated based on an established standard curve.

Determination of carotenoids. Carotenoids (lycopene, β-carotene, and lutein) were extracted using methods of Ishida et al. (2001) as modified by Simonne et al. (2007). Briefly, 5.0 g frozen tissues stored at –80 °C were thawed, weighed, mixed with 10 mL methylene chloride, and homogenized at 3600 g_n for 1 min. The solution was left to separate into distinct layers, and the bottom clear layer was collected into a 25-mL volumetric flask. This step was repeated several times to obtain a total of 25 mL clear solution, from which 10 mL was transferred to a beaker for drying. These samples were flush evaporated with nitrogen using an N-EVAP 116 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA). The dried residues were resuspended by adding a few drops of tetrahydrofuran. The solution was brought to a final 10-mL volume with 70:30 (v/v) methanol:methyl tert-butyl ether. Each carotenoid was identified and quantified using high-performance liquid chromatography according to the method of Simonne et al. (2007) [Water Alliance system 2695 Separation Module, 996 Photo-Diode-Array Detector, with an auto injector (injection volumes = 10 to 30 µL), and column temperature regulator; Waters Corporation, Milford, MA]. A reverse-phase C_{30} polymeric analytical column (ProntoSil C30, 250 × 4.6 mm i.d., 5-µm particle diameter; MAC-MOD Analytical, Inc., Chadds Ford, PA) was used for separations.

Determination of total phenolic content and antioxidant activity. Phenolic contents in the hydrophilic and lipophilic fractions of fruit tissues were quantified according to Toor and Savage (2005). In brief, 25.0 g homogenized fresh fruit tissue was weighed, centrifuged at 4 °C and 17,600 g_n for 20 min; and the supernatant was filtered through cheesecloth. The filtered supernatant was used to quantify the hydrophilic fraction of phenolics. For extraction of the lipophilic fraction, 25 mL acetone was added to the pellet from each sample after centrifugation and the mixture shaken for 1 h. The mixture was then centrifuged at 4 °C and 17,600 g_n for 20 min. The supernatant resulting from this second centrifugation was used to quantify the lipophilic fraction of total phenolics. Each extracted supernatant was diluted with deionized water, and triplicate, 0.4-mL aliquots of each extract was combined with 2.5 mL freshly diluted, 0.2% Folin–Ciocalteu reagent. This oxidation reaction was neutralized by adding 2.0 mL of 7.5% w/v saturated sodium

carbonate, and the samples were vortexed for 15 s. The reaction mixtures were subsequently incubated in a water bath at 45 °C for 20 min. A 250-µL sample of each reaction mixture was pipetted into the microplate and absorbance measured at 765 nm with a microplate spectrophotometer (BioTek). The phenolic content of each fraction was calculated using gallic acid as standard.

The same supernatants of the hydrophilic and lipophilic extracts described earlier were also used for assaying total antioxidant capacity as ORAC as described by Huang et al. (2002). For a standard, this assay uses Trolox® (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), which is a vitamin E analogue and known antioxidant. After diluting each fraction and standard with phosphate buffer (75 mM, pH 7.4), a 25-µL aliquot of each sample was mixed with 150 µL of 0.4 µM fluorescein solution in a microplate well. The plate with these mixtures was equilibrated by incubating for 15 min in a Synergy HT Multi-Detection Microplate Reader (BioTek). Scavenging reactions were then initiated by adding 25 µL of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) solution for a final volume of 200 µL. The fluorescence was monitored kinetically over a 35-min period. The antioxidant activity, i.e., the AAPH free radical scavenging capacity of each sample, was calculated relative to the Trolox standard. The inhibition capacity of each sample was converted to $\mu mol \cdot L^{-1}$ Trolox equivalent antioxidant capacity. All determinations were carried out in duplicate.

Statistical analyses. Data were analyzed using the Proc Glimmix program of the SAS package for Windows (version 9.2; SAS Institute Inc., Cary, NC) following the randomized complete block design used. Data from each quality attribute were analyzed with a model that included the main effects of year (season) and grafting, with interaction terms for the two main effects. Multiple comparisons among treatments were performed by Fisher's least significant difference test at $P \leq 0.05$.

Results

Moisture content, SSC, pH, and TTA. Fruit moisture content did not differ between the 2010 and 2011 growing seasons, but fruit from plants with the interspecific hybrid rootstocks consistently showed small, but significant increases in moisture (by 0.6% on average) relative to fruit from the non- and self-grafted control plants (Table 1). Also, except for SSC, no significant differences were observed for the quality attributes pH, TTA, and SSC:TTA ratio between fruit from control plants and those grafted onto hybrid rootstocks (Table 1). Values of the TTA ranged from 0.25% for the fruit from the plants grafted onto 'Beaufort' to 0.28% for the fruit from the self-grafted plants, with no significant differences among grafted and nongrafted plants. Interestingly, SSC values were greater for fruit from self-grafted plants in contrast to the nongrafted control and the treatments with hybrid rootstocks, whereas no significant difference was observed between nongrafted control and plants grafted with the hybrid rootstocks. In addition, no differences in SSC translated into any significant effects on the SSC:TTA ratios. The SSC:TTA ratios varied from 13.48 for fruit from the plants grafted onto 'Multifort' to 15.15 in the self-grafted plants.

Seasonal comparisons of these quality-related attributes showed that the TTA values were significantly higher during the 2010 season than during 2011, whereas the opposite trend was observed for pH and the SSC:TTA ratio values. Specifically, averaged over the four treatments, values of pH were 4.40 in 2010 and 4.50 in 2011, while TTA was 0.29% in 2010 and 0.24% in 2011. The SSC:TTA ratio was 13.26 in 2010 and 15.52 in 2011. In contrast, similar values of fruit moisture and SSC were observed between the two seasons.

Ascorbic acid and carotenoid contents. Levels of ascorbic acid were significantly lower in fruit from plants grafted onto the two interspecific hybrid rootstocks (Table 2), and averaged ≈22% less than the average of non- and self-grafted controls [17.2 vs. 21.9 mg/100 g fresh weight (FW)]. Interestingly, fruit from self-grafted plants showed a significantly higher level of ascorbic acid than the nongrafted control. Levels of lycopene in fruit were statistically similar among the four treatments, ranging from 20.0 to 22.7 µg/g FW over the two seasons (Table 2). Similarly, levels of β-carotene did not differ significantly in fruit from grafted plants compared with those of the self- and nongrafted plants. With

Table 1. Moisture, pH, total titratable acidity (TTA), soluble solids content (SSC), and their ratio (SSC:TTA) of tomato fruit from non- and self-grafted 'Florida 47' controls and grafted 'Florida 47' with interspecific hybrid rootstocks. Breaker stage fruit were harvested at 80 days after transplanting (DAT) in 2010 and 75 DAT in 2011, then allowed to ripen at 20 °C until fully red.

	Moisture (%)	pH	SSC (°Brix)	TTA (% citric acid)	SSC:TTA ratio
Season (S)					
2010	95.12 a	4.40 b	3.83 a	0.29 a	13.26 b
2011	94.90 a	4.50 a	3.75 a	0.24 b	15.52 a
P value	0.06	<0.01	0.62	0.01	<0.01
Grafting[z] (G)					
FL/BE	95.37 a	4.46 a	3.59 b	0.25 a	14.29 a
FL/MU	95.25 a	4.45 a	3.58 b	0.27 a	13.48 a
FL/FL	94.59 b	4.43 a	4.24 a	0.28 a	15.15 a
FL	94.83 b	4.45 a	3.75 b	0.26 a	14.65 a
P value	<0.01	0.535	0.004	0.187	0.253
S × G interaction	0.46	0.39	0.70	0.44	0.17

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ based on Fisher's least significant difference test.
[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL/FL = self-grafted 'Florida 47'; FL = nongrafted 'Florida 47'.

Table 2. Ascorbic acid and carotenoid contents of tomato fruit from non- and self-grafted 'Florida 47' controls and grafted 'Florida 47' with interspecific hybrid rootstocks. Breaker stage fruit were harvested at 80 days after transplanting (DAT) in 2010 and 75 DAT in 2011, then allowed to ripen at 20 °C until fully red.

	Ascorbic acid (mg/100 g FW)	Lycopene (µg/g FW)	β-carotene (µg/g FW)	Lutein (µg/g FW)	
Season (S)					
2010	21.5 a	15.4 b	4.1 a	—	
2011	17.6 b	27.0 a	2.4 b	—	
P value	0.01	<0.01	<0.01	<0.01	
Grafting[z] (G)				2010	2011
FL/BE	17.0 c	21.3 a	3.3 a	2.8 bA	1.0 aB
FL/MU	17.3 c	20.8 a	3.3 a	3.9 aA	0.8 aB
FL/FL	23.3 a	20.0 a	3.1 a	3.4 abA	0.9 aB
FL	20.5 b	22.7 a	3.2 a	3.4 abA	0.9 aB
P value	<0.01	0.56	0.87	0.18	
S × G interaction	0.51	0.17	0.43	0.03	

Means followed by the same lowercase letter within a column, and means followed by the same uppercase letter within a row are not significantly different at $P \leq 0.05$ based on Fisher's least significant difference test.
[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL/FL = self-grafted 'Florida 47'; FL = nongrafted 'Florida 47'.

respect to lutein, a significant grafting × year interaction was observed. In 2010, FL/MU had significantly higher lutein than FL/BE, but the level did not differ from the non- or self-grafted controls. However, no treatment differences for lutein were found in 2011. In this study, as expected, lycopene was the dominant carotenoid present, regardless of the grafting treatment and season.

Seasonal differences resulted in significantly higher contents of ascorbic acid, β-carotene, and lutein for all the treatments in the 2010 growing season, whereas lycopene content was higher in 2011 than in 2010 by 75% (Table 2).

Total phenolic content and antioxidant capacity. Total phenolics were predominantly hydrophilic types, which were consistently over 4-fold more abundant than the lipophilic phenolics, irrespective of the growing season or grafting treatment (Table 3). The only detectable difference among treatments in total phenolics or their fractions was a lesser amount of lipophilic phenolics in the fruit of plants grafted with 'Multifort' (FL/MU) compared with those of the nongrafted plants (FL). In terms of antioxidant capacity, the hydrophilic ORAC values of tomato fruit did not differ significantly between grafted and nongrafted plants. By contrast, the lipophilic ORAC values were consistently lower in the fruit from plants with the two hybrid rootstocks (FL/BE and FL/MU) relative to those of the fruit from self- and nongrafted controls while FL/BE exhibited a higher value than FL/MU (Table 3). A significant season by grafting interaction effect was observed for total antioxidant capacity, with tomato plants grafted onto 'Multifort' showing higher ORAC values in the fruit than for the nongrafted control in the 2011 season, but ORAC values were similar among treatments in 2010.

Hydrophilic, lipophilic, and total phenolic contents were significantly higher in 2011 than those in 2010. However, the antioxidant activity measured as hydrophilic, lipophilic, and total ORAC values did not vary significantly between the two production seasons.

Yield and yield components. Total and marketable fruit yields averaged over the two seasons were significantly higher in grafted plants with the interspecific hybrid rootstocks than non- and self-grafted plants (Table 4). On average, grafting with the hybrid rootstocks increased total and marketable fruit yields by 36% and 41%, respectively. The yield improvement could be attributed to a significantly higher number of fruit per plant and greater average fruit weight. The total and marketable fruit numbers were 18% and 25% greater, respectively, for plants with hybrid rootstocks than for non- and self-grafted controls. Similarly, the average weight of single tomato fruit for marketable yield was greater in plants grafted with hybrid rootstocks by 13% in comparison with non- and selfgrafted plants. The tomato fruit yields from the two seasons were similar, with the exception of greater marketable fruit yields and fruit number per plant in 2011 (Table 4).

Discussion

Work here shows that grafting with vigorous rootstocks can enhance fruit yield and yield components of tomato plants without major changes to fruit quality. The increased yield demonstrated in this study is consistent with previous reports on grafted tomatoes across various environmental conditions in both field and greenhouse systems (Fernandez-Garcia et al., 2004; Leonardi and Giuffrida, 2006; O'Connell et al., 2012; Rivard et al., 2010). As shown in this study and the work by others, the increase in total yield of grafted vs. non- and/or self-grafted plants often resulted from the increase in average fruit weight and/or fruit number. Physiologically, the improved growth parameters of grafted plants may be related to an increase in water and nutrient uptake compared with the self-rooted plants, possibly owing to the larger and vigorous root system of the rootstock (Martínez-Ballesta et al., 2010). In addition, growth and yield enhancement in grafted plants is also attributed to a greater

Table 3. Total phenolic content and antioxidant capacity (as assayed by oxygen radical absorbance capacity) in tomato fruit from non- and self-grafted 'Florida 47' controls and grafted 'Florida 47' with interspecific hybrid rootstocks. Breaker stage fruit were harvested at 80 days after transplanting (DAT) in 2010 and 75 DAT in 2011, and then allowed to ripen at 20 °C until fully red.

	Total phenolics (mg GAE/100 g FW)[y]			Antioxidant capacity (μmol TEAC/100 g FW)[x]			
	Hydrophilic	Lipophilic	Total	Hydrophilic	Lipophilic	Total	
Season (S)							
2010	11.0 b	2.3 b	13.2 b	277.2 a	66.4 a	343.6 a	
2011	14.5 a	3.3 a	17.7 a	262.7 a	61.8 a	324.5 a	
P value	<0.01	<0.01	<0.01	0.62	0.11	0.53	
Grafting[z] (G)						2010	2011
FL/BE	12.9 a	2.6 ab	15.5 a	263.6 a	61.7 b	351.9 a	298.6 b
FL/MU	13.7 a	2.4 b	16.1 a	302.9 a	54.5 c	319.8 a	395.1 a
FL/FL	12.5 a	2.8 ab	15.4 a	287.6 a	69.6 a	382.3 a	331.9 ab
FL	11.8 a	3.2 a	14.9 a	225.7 a	70.6 a	320.3 a	272.3 b
P value	0.59	0.05	0.87	0.04	<0.01	0.08	
S × G interaction	0.35	0.40	0.33	0.09	0.10	0.05	

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ based on Fisher's least significant difference test.
[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL/FL = self-grafted 'Florida 47'; FL = nongrafted 'Florida 47'.
[y]GAE = Gallic acid equivalents.
[x]TEAC = Trolox equivalent antioxidant capacity.

Table 4. Total yield, marketable yield, fruit number, and average fresh weight of fruit from non- and self-grafted 'Florida 47' controls and grafted 'Florida 47' with interspecific hybrid rootstocks.

	Total yield		Marketable yield		
	Yield (Mg/ha)	Fruit number per plant	Yield (Mg/ha)	Fruit number per plant	Avg fruit wt (g/fruit)
Season (S)					
2010	54.9 a	22.1 a	48.2 b	17.4 b	191.6 a
2011	59.7 a	23.6 a	54.9 a	20.2 a	186.4 a
P value	0.27	0.50	0.13	0.09	0.36
Grafting[z] (G)					
FL/BE	66.1 a	24.4 ab	60.7 a	21.0 a	201.5 a
FL/MU	65.8 a	25.0 a	59.9 a	20.8 a	200.2 a
FL/FL	48.3 b	20.6 c	42.9 b	16.7 b	177.9 b
FL	48.8 b	21.3 bc	42.7 b	16.8 b	176.5 b
P value	<0.01	<0.01	<0.01	<0.01	<0.01
S × G interaction	0.46	0.91	0.26	0.55	0.43

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ based on Fisher's least significant difference test.
[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL/FL = self-grafted 'Florida 47'; FL = nongrafted 'Florida 47'.

functional relationship between the rootstock and the scion in terms of hormones, proteins, and other metabolites (Aloni et al., 2010; Harada, 2010). In this study, fruit yields were similar for self-grafted (FL/FL) and nongrafted (FL) plants, thus reinforcing the fact that yield increase was directly the result of the specific rootstocks used rather than the grafting process per se.

In addition to showing a clear enhancement of yield of tomato plants by grafting with vigorous rootstocks, we show that this can be achieved without major changes to nutritional quality attributes of the tomato fruit. Slight increases in fruit moisture content were observed for fruit from plants grafted onto hybrid rootstocks compared with those of non- and self-grafted control plants, but the difference was only ≈0.6%. This small increase in fruit moisture content is consistent with observations by Turhan et al. (2011) who found that fruit dry matter decreased by 0.4% to 0.6% in three different tomato cultivars grafted onto 'Beaufort' and 'Arnold' rootstocks in comparison with nongrafted plants in a greenhouse study. The dry matter reductions were accompanied by 9.5% to 10.5% decreases in SSC (Turhan et al., 2011). Consistent with data reported in the current study, Schwarz et al. (2013) also found a 0.4% increase in the fruit water content of 'Piccolino' tomato grafted onto 'Maxifort' rootstock compared with the fruit from self-grafted 'Piccolino'.

Perception of sweetness and sourness of tomato fruit is related to its SSC and TTA, and the ratio between SSC and TTA often defines the fruit flavor level (Georgelis et al., 2004; Tigist et al., 2013). In addition, high values for both SSC and TTA are desirable, especially when the tomato fruit are produced for fresh-market consumption (Cuartero and Fernández-Muñoz, 1999; Saltveit, 2005). In the present work, with the growing conditions and treatments described, the SSC:TTA ratios of the fruit did not differ significantly. Results for TTA and SSC have varied in previous studies concerning rootstock effects. Enhancement of fruit TTA (Flores et al., 2010; Schwarz et al., 2013; Turhan et al., 2011) and SSC (Flores et al., 2010) in certain rootstock–scion combinations has been reported, while decreases in SSC as a result of grafting were also noted (Nicoletto et al., 2013; Pogonyi et al., 2005; Schwarz et al., 2013; Turhan et al., 2011), and still others have observed no rootstock effect on SSC, TTA, and SSC:TTA ratio in tomato fruit (Barrett et al., 2012b; Di Gioia et al., 2010). Accumulation of water in tomato fruit can lead to a decrease in the concentration of sugars and acids in fruit juice (Balibrea et al., 2006). In the present study, the higher fruit moisture content in fruit from hybrid rootstock grafted plants could have diluted soluble solids to some degree, but the effect would probably have been minimal since the SSC reduction in FL/BE and FL/MU was only in contrast to the self-grafted plants rather than the nongrafted control. The dilution effect was also previously reported for citrus fruits from trees with more vigorous rootstocks. For example, Albrigo (1977) demonstrated that fruit from orange trees grafted on rough lemon (vigorous rootstock) had the lowest SSC and the highest leaf water potentials. Furthermore, in tomato, a negative correlation between fruit size and sugar concentration has also been reported (Georgelis et al., 2004). As shown in our work and previous studies by others (Turhan et al., 2011), grafting with specific rootstocks can enhance tomato fruit yield and average fruit weight. Therefore, some degree of dilution-related decrease in the SSC values might be expected when grafting increases fruit size, but was minimal here.

Effects of grafting on fruit SSC may extend to sugar components such as levels of the reducing sugars, especially glucose and fructose, but further research will be needed to address this question. Grafting was reported to reduce the total sugar concentration of tomato fruit by 22% to 53%, with the effect differing significantly between the rootstocks used (Turhan et al., 2011). Other studies have reported that the highest levels of glucose and fructose occurred in fruit from grafted tomato plants using drought-tolerant rootstocks under stress conditions (Sanchez-Rodriguez et al., 2012). A more complete analysis should also include assays of enzymes for sucrose metabolism and translocation (e.g., invertase, sucrose synthase, and sucrose phosphate synthase) that are involved in the accumulation of sugars in tomato fruit. Such analyses would help determine whether any variation in the enzyme activities could be attributable to grafting with specific rootstocks. Molecular marker–assisted breeding techniques (Tieman et al., 2012) could help further explore the genetics and biochemistry of flavor in an array of wild tomato species that could be used to develop tomato rootstocks that help maintain and improve fruit eating quality in addition to growth, yield enhancement, and pathogen resistance.

Additionally, tomato fruit quality is also related to the levels of various antioxidants, including ascorbic acid, carotenoids, and phenolic compounds present in the fruit. Results here and elsewhere indicate that rootstocks have inconsistent effects on these attributes. With regard to vitamin C, study on heirloom tomato 'Brandywine' did not show any significant change in fruit vitamin C content when plants were grafted onto two different types of rootstocks 'Multifort' and 'Survivor' (Barrett et al., 2012b) while Sanchez-Rodriguez et al. (2012) observed higher levels of vitamin C in tomato fruit when plants were grafted onto drought-tolerant rootstock, and Chávez-Mendoza et al. (2013) measured an increase of 3.9% in vitamin C in bell pepper fruit from grafted plants compared with nongrafted plants. However, in line with findings from this work, reduced levels of vitamin C have also been observed in grafted tomatoes by others. Di Gioia et al. (2010) observed a grafting-based decline in vitamin C content of 'Cuore di Bue' tomato fruit that persisted throughout various harvest dates. Vinkovic Vrcek et al. (2011) suggested that a significant decrease in total vitamin C content of tomato fruit from grafted plants may result from redistribution or accumulation of vitamin C in other plant parts and may also be associated with the greater accumulated biomass in the shoot of grafted plants. From a nutritional standpoint, the relatively slight decrease in vitamin C content due to grafting may in fact have minimal implication on the nutritional contribution of tomato fruit because fresh tomatoes, in general, are the second most important source of vitamin C after orange juice (Gahler et al., 2003). Also, according to Rickman et al. (2007), despite the heat-sensitive nature of vitamin C, the processed tomato can still be significant in meeting the Recommended Daily Allowance in vitamin C.

Unlike the modest reduction in ascorbic acid content, grafting with vigorous rootstocks led to no apparent change in the levels of fruit carotenoids, especially lycopene and β-carotene. Other researchers have reported an increase in the carotenoid content (lycopene, β-carotene) in fruit of grafted tomato (Fernandez-Garcia et al., 2004; Sanchez-Rodriguez et al., 2012), and the rootstock–scion interaction effects on tomato carotenoids (Brajović et al., 2012; Schwarz et al., 2013). Some rootstocks may have a positive influence on the processes leading to biosynthesis and accumulation of carotenoids, especially lycopene, in tomato fruit. Carotenoid synthesis during the ripening of tomato fruit involves a progressive transformation of chloroplasts into chromoplasts; as photosynthetic membranes are being degraded and chlorophyll metabolized, leading to the accumulation of carotenoids, including β-carotene and lycopene (Saltveit, 2005).

Potassium (K) is required for protein synthesis and activity of acetic thiokinase, the enzyme directly involved in the biosynthesis of lycopene (Taber et al., 2008). One suggestion is that the increase in lycopene synthesis and accumulation in tomato fruit may be in part related to the high K concentration in the fruit (Fanasca et al., 2006), although the relation of K to tomato lycopene enhancement was also found to be cultivar specific (Taber et al., 2008). Also, the enhanced nutrient uptake by grafted plants might lead to greater chlorophyll content in the green, photosynthesizing fruit, which in turn could produce more carotenoids on degradation. However, regulatory mechanisms that control biosynthesis and accumulation of carotenoids are complex and not well understood (Bramley, 2002).

In this study, the amount of total phenolics, measured as the sum of the hydrophilic and lipophilic phenolic fractions, remained constant in tomato fruit from grafted and nongrafted plants. These results do not agree with those reported by Vinkovic Vrcek et al. (2011), who observed a decrease in total phenolics in fruit of grafted tomato plants with three different commercial rootstocks, including 'Efialto', 'Heman', and 'Maxifort'. Similarly, Chávez-Mendoza et al. (2013) noted that total phenolic content of bell pepper fruit from grafted plants was more than 6% lower than

that of the fruit from nongrafted plants. The impact of grafting on phenolic acid content in tomato fruit was also reported to vary with different rootstocks (Nicoletto et al., 2013). In the present study, despite the lower lipophilic antioxidant capacity of fruit as a result of grafting with the two interspecific rootstocks, total antioxidant capacity of fruit was not significantly affected by grafting except for the higher ORAC values found in FL/MU compared with nongrafted plants. Our results differed from those of Vinkovic Vrcek et al. (2011), who reported significantly lower levels of antioxidant activity along with the lower total phenolics in fruit of grafted plants. However, we assayed total hydrophilic and lipophilic antioxidant activity via ORAC as opposed to the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay in their study. Significant differences in antioxidant activity measurements with different methods have been reported (Corral-Aguayo et al., 2008; Ou et al., 2002).

Nutritional quality of tomato fruit is typically subjected to variations in environmental conditions (Scott, 2002). Oftentimes, fruit of the same tomato cultivar may vary in quality depending on production conditions. Such effects could account for the significant differences observed in many fruit quality attributes between the 2010 and 2011 production seasons in this study. At the research site, the 2010 Spring growing season turned out to have a considerably higher level of precipitation than in 2011 (Djidonou et al., 2013). Moreover, variations noticed in our results relative to those presented in the literature could be explained by the genotype × environment interactions, which often greatly influence the nutritional quality (Panthee et al., 2012; Roselló et al., 2011). Environmental factors most often implicated include soil, temperature, light intensity, humidity, rainfall, and photoperiod (Beckles, 2012). In addition, rootstock effects on fruit quality can be influenced by cultural practices (e.g., soilless vs. soil culture, irrigation, and fertilization), type of scion–rootstock combinations used, and harvest date (Davis et al., 2008). Flores et al. (2010) speculated that in grafted plants, metabolic processes inherent to fruit quality are generally species driven and are largely controlled by the scion. However, some of the fruit quality attributes may also be influenced by the rootstock as a result of metabolites, hormones, mobile RNAs, or other signals moving from root to scion through xylem, and/or changes in the physiological processes of the scion (Lee, 1994; Melnyk and Meyerowitz, 2015; Rouphael et al., 2010). Analysis of gene expression, mobile RNAs, and/or metabolic profiles may provide additional insights into some of the changes in nutritional quality related to grafting with interspecific hybrid rootstocks.

Conclusions

Overall, comparative analysis of tomato quality in response to grafting using determinate tomato 'Florida 47' as the scion showed that the increased yields achieved by grafting with interspecific rootstocks were not accompanied by pronounced changes in the compositional parameters of fruit measured in this 2-year study. Seasonal effects outweighed the rootstock effects on fruit quality attributes. The major consistent rootstock effects on fruit quality were found in fruit moisture and ascorbic acid as shown by 0.6% greater fruit moisture content and 22% less ascorbic acid in fruit from grafted plants in comparison with non- and self-grafted plants on average. Compared with nongrafted tomato plants, grafted plants with the two interspecific hybrid rootstocks demonstrated comparable levels of soluble solids, titratable acidity, carotenoid and phenolic contents, and antioxidant capacity. Nevertheless, whether the grafting process with different rootstocks has an additional effect on fruit quality beyond the chemical composition deserves more in-depth research, especially in terms of potential genetic and biochemical contributions to tomato flavor.

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