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Nitrogen Accumulation and Root Distribution of Grafted Tomato Plants as Affected by Nitrogen Fertilization

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Abstract. **Growth and yield typically increase when tomato plants are grafted to selected interspecific hybrid rootstocks from which distinctive root system morphologies are envisioned to aid nutrient uptake. We assessed these relationships using a range of exogenous nitrogen (N) supplies under field production conditions. This study analyzed the impact of N on growth, root distribution, N uptake, and N use of determinate 'Florida 47' tomato plants grafted onto vigorous, interspecific, hybrid tomato rootstocks 'Multifort' and 'Beaufort'. Six N rates, 56, 112, 168, 224, 280, and 336 kg·ha^{-1}, were applied to sandy soil in Live Oak, FL, during Spring 2010 and 2011. During both years, the leaf area index, aboveground biomass, and N accumulation (leaf blade, petiole, stem, and fruit) responded quadratically to the increase in N fertilizer rates. Averaged over the two seasons, the aboveground biomass, N accumulation, N use efficiency (NUE), and N uptake efficiency (NUpE) were ≈29%, 31%, 30%, and 33% greater in grafted plants than in nongrafted controls, respectively. More prominent increases occurred in the root length density (RLD) in the uppermost 15 cm of soil; for grafted plants, RLD values in this upper 15-cm layer were significantly greater than those of nongrafted plants during both years with an average increase of 69% over the two seasons. Across all the grafted and nongrafted plants, the RLD decreased along the soil profile, with ≈60% of the total RLD concentrated in the uppermost 0 to 15 cm of the soil layer. These results demonstrated a clear association between enhanced RLD, especially in the upper 15 cm of soil, and improvements in tomato plant growth, N uptake, and N accumulation with grafting onto vigorous rootstocks.**

For most crop species, nitrogen (N) is an essential plant nutrient with the greatest influence on growth and development because it is a constituent of chlorophyll, amino acids, proteins, nucleic acids, and cell walls (Fageria, 2009). Because N fertilization is critical for optimal shoot and root growth, it is often applied with the greatest quantity and frequency in many production systems of high-value crops like tomato (*Solanum lycopersicum*) (Hartz and Bottoms, 2009). However, the high mobility of N in the soil profile, together with the high requirement of N by crops, has led to N fertilization practices that cause environmental concerns (Vázquez et al., 2006; Zotarelli et al., 2009b). Best management practices (BMPs) have been developed to improve fertilizer use efficiency by plants and minimize the adverse impact of nutrient loss from the production site. On sandy soils with low intrinsic water and nutrient retention capacities, site-specific practices are being used to increase the nutrient residency time in the root zone. Popular solutions among vegetable growers include fertigation with drip irrigation, controlled-release fertilizer, crop rotation, cover cropping, and soil moisture–sensing methods (Simonne et al., 2017). In addition to improving management practices, the selection and use of genotypes with inherently high N use efficiency (NUE) can reduce the N fertilizer requirement, mitigate environmental concerns associated with N losses in the production system, and help ensure stability of high yields (Lynch, 1998). Crop genotypes with improved physical root traits may increase nutrient uptake and yield, especially in low-fertility soils (Rengel and Marschner, 2005), and nutrient-efficient germplasm has been explored as an important component of integrated nutrient management (Lynch, 1998; Wiesler et al., 2001).

Alternatively, vegetable grafting with interspecific hybrid rootstocks provides a viable option that has been demonstrated to improve crop nutrient use efficiency (Djidonou et al., 2013). At first, this approach was used primarily as an effective tool to manage various soil-borne diseases and to overcome environmental stresses associated with the intensive, continuous cropping in solanaceous and cucurbitaceous vegetable production systems (Lee et al., 2010). More recent work has shown that grafting with vigorous rootstocks can enhance nutrient uptake (Nawaz et al., 2016) and water use efficiency (Djidonou et al., 2013; Rouphael et al., 2008; Suchoff et al., 2018b). Depending on production conditions and scion–rootstock interactions, grafted tomato plants can also increase marketable fruit yield by 20% to 62% compared to nongrafted plants (Di Gioia et al., 2010; Djidonou et al., 2013; Lee and Oda, 2003; Leonardi and Giuffrida, 2006; Pogonyi et al., 2005). Similar grafting effects on yield improvement have been reported for cucurbit crops such as melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*). For example, Proietti et al. (2008) reported that the total and marketable yields of mini watermelon could be increased by 46% and 64%, respectively, if grafted onto selected rootstocks. Grafting 'Minirossa' mini watermelon onto 'Vita' rootstock also increased NUE (yield/applied N rate), N uptake efficiency (NUpE) (plant N content/applied N rate), and physiological N utilization efficiency (yield/plant N content) by 38%, 21%, and 17%, respectively (Colla et al., 2011). This improved efficiency of nutrient uptake and use in grafted plants may be related to the enhanced root size, architecture, and distribution for selected rootstocks. Root characteristics that may contribute to nutrient and water uptake include root length, root density, number and length of root hairs, root surface area, and intrinsic uptake capacity (Martínez-Ballesta et al., 2010). The improved root development observed for grafted plants has been reported for hydroponics but not under field conditions. Tomato root density and number of root hairs were significantly improved when grafted plants were compared with self-grafted plants grown in perlite substrate (Oztekin et al., 2009). Using a greenhouse pot study, Suchoff et al. (2018a) also detected a significant increase in total root length and more finer roots in tomato plants grafted with 'Beaufort' than the self-grafted control. Conversely, Miller et al. (2013) did not find differences in root length density (RLD) between grafted and nongrafted watermelon during a 3-year field study.

To date, few systematic studies have addressed root distribution characteristics of grafted tomato plants relative to nongrafted controls under field conditions or in relation to soil N availability. Hence, the objective of the present study was to assess the growth, plant N concentration, N accumulation, N uptake, NUE, and root distribution of grafted and nongrafted tomato plants under different N fertilization rates in sandy soils.

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Materials and Methods

Experimental site and design. Field experiments were conducted during Spring 2010 and 2011 at the University of Florida's North Florida Research and Education Center in Suwannee Valley in Live Oak, FL. The soil type is a Blanton-Foxwort-Alpin Complex sandy soil (Natural Resources Conservation Service, 2006). Detailed information about the grafted transplant production, field preparation, and management practices was provided by Djidonou et al. (2013). Briefly, a split-plot design with the whole plots arranged in a randomized complete block design with four replications was used during both years. The whole-plot treatments consisted of six N fertilizer application rates, 56, 112, 168, 224, 280, and 336 kg·ha^{-1} N, which represented 25%, 50%, 75%, 100%, 125%, and 150%, respectively, of the total N application rate recommended (224 kg·ha^{-1} N) by the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) for field production of irrigated round tomato in sandy soils in Florida (Olson et al., 2010). Irrigation was maintained at the level recommended by the UF/IFAS (0.9 to 3.7 mm·d^{-1} depending on the growth stage) for irrigation and field production of round tomato in sandy soils in Florida (Olson et al., 2010). The subplot involved three levels of grafting treatments: determinate tomato 'Florida 47' (Seminis Vegetable Seeds, Inc., St. Louis, MO) grafted onto 'Beaufort' (FL/BE); 'Florida 47' grafted onto 'Multifort' (FL/MU); and the nongrafted 'Florida 47' (FL) as the control. All were randomized within each whole plot. Both 'Beaufort' and 'Multifort' (De Ruiter Seeds Inc., Bergschenhoek, The Netherlands) are vigorous, interspecific tomato hybrid rootstocks (*S. lycopersicum* × *S. habrochaites*). There were 12 plants for each treatment combination per replication. Beds were 0.71 m wide and spaced 1.52 m apart (between the centers of two adjacent beds), with 0.46-m in-row spacing for open-field tomato production. Except for the 56 kg·ha^{-1} N treatment, which only included a preplant application of 13N–1.7P–10.8K, ammonium nitrate (34N–0P–0K; Mayo Fertilizer Inc, Mayo, FL) was injected weekly through the drip tape starting 1 week after transplanting to provide the remaining amount of N of each fertilization rate. Potassium chloride (Dyna Flo 0–0–15; Chemical Dynamics Inc, Plant City, FL) was also applied through fertigation to provide an equivalent supply of potassium for each N rate of treatments based on the soil test. Other cultural practices, including disease and pest control, followed the recommendations for commercial field tomato production in Florida (Olson et al., 2010).

Plant growth and N accumulation. Aboveground biomass was destructively evaluated on one representative plant per treatment combination in each replication at 85 d after transplanting (DAT) in 2010, and at 82 DAT in 2011, during the harvest period when the fruit load was fully developed. Each sampled plant was cut at the ground base and separated into the leaf blade, petiole, stem, and fruit, and the fresh weight of each plant part was recorded. Representative samples (≈300 g each) from the leaf blade, petiole, and stem and a sample of fruit (≈500 g) were taken from each sampled plant and weighed. Leaf area was measured with a LI-COR 3100 leaf area meter (LI-COR Inc., Lincoln, NE), and the leaf area index (LAI; m^2 leaf/m^2 land) was calculated. All subsamples from each plant were dried in a forced-air drying oven at 60 °C for 72 to 120 h until constant weight was achieved. Then, the total aboveground biomass was determined.

Dried subsamples of leaf blade, petiole, stem, and fruit were analyzed to determine the total Kjeldahl nitrogen concentrations using the combustion technique (O'Dell, 1993). Shoot N accumulation was determined by multiplying the dry mass of the leaf blade, petiole, stem, and fruit by the corresponding N concentrations. In addition, NUpE expressed as total N accumulated in the aboveground tissues (leaf blade, petiole, stem, and fruit) divided by the N supply and NUE as the ratio between total aboveground biomass and N supply were also estimated.

Root analysis. At 93 DAT in 2010, and at 97 DAT in 2011, root samples were collected from the grafted and nongrafted plants. The root analysis was performed using three N application rates, 112, 224, 336 kg·ha^{-1} N, following the root sampling method previously described by Zotarelli et al. (2009a). Briefly, roots were sampled by taking soil cores at four different depths, 0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm, using a 5-cm diameter soil auger and at two different positions around the plant (i.e., at the plant base vs. 15 cm from the plant) in the center of each treatment plot. Soil samples were stored at 4 °C before processing in the laboratory. During sample processing, each sample was weighed and washed with running water using a fine sieve to collect the root and other debris. Then, cleaned materials above the sieve were placed in a clear glass pan and tomato roots were carefully handpicked with tweezers and placed in petri dishes. Washed and cleaned roots per soil core were then scanned using a root scanning apparatus (EPSON color image scanner LA1600$^+$; EPSON, Toronto, Canada) and analyzed with image analysis software (WinRhizo 2008a; Regent Instruments, Quebec, Canada) to determine the total root length and total root surface area. The RLD (cm·cm^{-3}) for each soil depth was estimated as the total root length (cm) per volume of soil (cm^{-3}) of each soil depth. Also, the root surface area density (RSAD; cm^2·cm^{-3}) was calculated as the total root surface area (cm^2) per volume of soil (cm^{-3}).

Statistical analyses. Statistical analyses were performed using the GLIMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC). Due to a greater variation in the rainfall pattern between the two growing seasons (Djidonou et al., 2013), data for each season were analyzed separately. Within each season, aboveground biomass, plant N concentrations and accumulation, NUE, and NUpE were analyzed with a model including the main effects of the N rate and grafting as well as their interaction. Orthogonal polynomial contrasts (linear, quadratic, or cubic) were also used to evaluate the type of response to N fertilizer rate when the N rate effect was significant. RLD and RSAD were analyzed with a heteroscedastic linear mixed effects model including the main effects, two-way interactions, three-way interactions, and four-way interactions of the four factors of N rates, grafting, sampling position, and soil depth. Block and N rate within block were separately added to the models as random effects. However, models with block as a random effect led to smaller values of Akaike's information criterion that were considered for the analysis. The covariance structure with the residual option based on the effect of "sampling position × soil depth" was also specified in the models. Studentized residual plots were used to check normality and homogeneity of residuals, which determined that data transformations were not necessary in this study. Furthermore, whenever the F-test results for fixed effects were significant, a Tukey's test was performed for multiple comparisons of measurements among treatments ($P \leq 0.05$).

Results

Leaf area index and plant biomass. Both N rate and grafting significantly affected the LAI and aboveground plant biomass, but there was no significant interaction effect. The N rate also had quadratic effects on these two growth parameters (Table 1). In 2010, increasing N rates from 56 to 224 kg·ha^{-1} resulted in an increase in LAI and aboveground biomass (leaf blade, petiole, stem, and fruit). However, N rates of 280 and 336 kg·ha^{-1} did not significantly increase LAI or aboveground biomass accumulation compared with the recommended rate. In 2011, increasing N rates from 56 to 168 kg·ha^{-1} led to an increase in LAI and aboveground biomass, whereas higher N rates did not significantly increase aboveground biomass accumulation compared with the N rate of 168 kg·ha^{-1}. The LAI did not differ significantly between the N rates of 168 and 224 kg·ha^{-1}, but increasing N rates from 168 kg·ha^{-1} to 280 and 336 kg·ha^{-1} significantly increased the LAI. Grafted plants on either of the two rootstocks showed significantly higher levels of LAI and aboveground biomass compared with nongrafted plants, and both FL/BE and FL/MU performed similarly. Averaged over the N rates, grafting with the two rootstocks increased LAI by ≈33% and 35% in 2010 and 2011, respectively (Table 1). This enhancement in LAI was accompanied by improved dry matter accumulation; i.e., the total aboveground biomass of grafted plants was greater than that of

Table 1. Effects of the nitrogen (N) fertilization rate and grafting with 'Multifort' and 'Beaufort' rootstocks on leaf area index (LAI), aboveground biomass, N accumulation, N use efficiency (NUE), and N uptake efficiency (NUpE) of 'Florida 47' tomato plants during the 2010 and 2011 field trials in Live Oak, FL.

Treatment	LAI ($m^2 \cdot m^{-2}$)	Aboveground biomass[z] ($Mg \cdot ha^{-1}$)	N accumulation[y] ($kg \cdot ha^{-1}$)	NUE ($kg \cdot ha^{-1}$)	NUpE (%)
			2010		
N rate ($kg \cdot ha^{-1}$)					
56	0.74 c	1.85 c	43.29 c	33.09 a	77.3 a
112	1.84 b	2.72 c	65.61 c	24.31 b	58.6 bc
168	2.48 b	4.04 b	101.21 b	24.06 b	60.2 bc
224	3.75 a	5.61 a	146.48 a	25.08 b	65.4 ab
280	3.84 a	5.75 a	144.32 a	20.55 bc	51.5 bc
336	4.28 a	6.12 a	166.50 a	18.23 c	49.6 c
Contrast[x]	Q**	Q**	Q**	L***	L***
Graft[w]					
FL/BE	3.07 a	4.48 a	114.77 a	24.93 a	62.5 a
FL/MU	3.09 a	4.56 a	118.19 a	25.96 a	65.3 a
FL	2.31 b	4.01 b	100.74 b	21.77 b	53.5 b
			2011		
N rate ($kg \cdot ha^{-1}$)					
56	0.44 d	1.32 c	28.17 d	23.53 c	50.3 b
112	1.83 c	3.95 b	85.27 c	35.31 a	76.1 a
168	2.75 bc	4.96 ab	114.79 bc	29.51 b	68.3 a
224	3.46 ab	4.99 a	114.83 bc	22.29 cd	51.2 b
280	4.13 a	5.61 a	138.06 ab	20.04 cd	49.3 b
336	4.22 a	5.85 a	145.46 a	17.41 d	43.3 b
Contrast[x]	Q***	Q***	Q***	L***	L***
Graft[w]					
FL/BE	2.96 a	4.47 b	105.73 b	24.77 b	57.0 b
FL/MU	3.18 a	5.38 a	127.15 a	29.37 a	67.1 a
FL	2.27 b	3.48 c	80.41 c	19.91 c	45.3 c

[z]Shoot and fruit were included in the determination of total aboveground biomass.
[y]Total N accumulation in shoot and fruit.
[x]Linear (L), quadratic (Q), and cubic (C) polynomial contrasts were performed; *, **, ***significant at $P \leq 0.05$, 0.01, or 0.001, respectively. When significant L and Q contrasts were observed, only Q was reported.
[w]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL = nongrafted 'Florida 47'.
NUE is measured as the ratio of total biomass of the aboveground tissues to the amount of N applied ($kg \cdot ha^{-1}$).
NUpE is measured as the percentage of total N accumulated in the aboveground tissues compared to the amount of N applied ($kg \cdot ha^{-1}$).
Means within a column followed by the same letters do not differ significantly at $P \leq 0.05$ according to Tukey's test.

nongrafted controls by ≈13% and 42% in 2010 and 2011, respectively.

N accumulation, NUE, and NUpE. The N fertilizer rate and grafting each significantly enhanced the accumulation of plant N during both seasons, but no significant two-way interaction was evident. There was a significant quadratic response of the accumulated plant N to the N application rate during both years (Table 1). In 2010, increasing N rates from 56 to 224 $kg \cdot ha^{-1}$ consistently improved N accumulation in the aboveground tissues from 43.29 to 146.48 $kg \cdot ha^{-1}$. Increasing the N rate to 336 $kg \cdot ha^{-1}$ did not lead to additional increases in the accumulated plant N. During the 2011 season, accumulated N increased from 28.17 to 114.79 $kg \cdot ha^{-1}$ as the N rate increased from 56 to 168 $kg \cdot ha^{-1}$, whereas similar values were observed between 168 and 224 $kg \cdot ha^{-1}$. However, greater increases of the N rate to 336 $kg \cdot ha^{-1}$ did enhance the accumulated plant N relative to the 224 $kg \cdot ha^{-1}$. During both seasons, N accumulation was significantly greater in grafted plants compared with nongrafted controls. Results of grafting treatments were similar for both rootstocks in 2010; however, in 2011, FL/MU accumulated more N than did FL/BE. When N fertilization rates and grafting treatments were averaged, the accumulated N in grafted plants increased by ≈16% and 45% relative to nongrafted controls in 2010 and 2011, respectively (Table 1).

The effects of the N rate on both NUE and NUpE showed a negative linear trend (Table 1). During both seasons, NUE and NUpE linearly decreased as the N rate increased. Overall, the percentage of N uptake ranged from 77.3% with the 56 $kg \cdot ha^{-1}$ N rate to 43.3% with the 336 $kg \cdot ha^{-1}$ N rate. Regarding the grafting effect on these N use parameters, the results slightly varied with the growing season. In 2010, FL/BE and FL/MU exhibited similar impacts in contrast to the nongrafted control, and NUpE and NUE were significantly improved by an average of 19% and 17%, respectively. However, in 2011, the rootstocks performed differently, with FL/MU significantly increasing NUpE and NUE by ≈48% and FL/BE resulting in a significant increase in NUpE and NUE by ≈26% and 24%, respectively, compared with nongrafted plants (Table 1).

Analysis of plant tissue N concentrations revealed greater effects of the N application rate than grafting with vigorous rootstocks. The N fertilizer rates significantly increased N concentrations in the plant tissues (leaf blades and stems) with a linear effect; the highest N concentrations resulted from the highest N rates during both years (Table 2). During the 2010 season, the leaf blade N concentration at 336 $kg \cdot ha^{-1}$ N did not differ significantly from that at 224 or 280 $kg \cdot ha^{-1}$ N, whereas similar leaf blade N concentrations were found among N rates more than 56 $kg \cdot ha^{-1}$ N in 2011. The stem N concentration at 336 $kg \cdot ha^{-1}$ N was similar to that at 224 or 280 $kg \cdot ha^{-1}$ N in 2010 and that at 280 $kg \cdot ha^{-1}$ N in 2011. The fruit N concentration was also significantly impacted by N fertilization in 2010. In contrast, grafting only showed an effect on the leaf petiole N concentration in 2010 (Table 2).

Root length density. During the two seasons, both the N rate and grafting enhanced RLD, especially in the uppermost soil layer (Tables 3–5). Increasing the N rate from 112 to 224 $kg \cdot ha^{-1}$ increased the RLD by ≈17% and 69% in 2010 and 2011, respectively (Fig. 1). However, the increase was only significant during the 2011 season. When the treatments were averaged, the total RLD was 16% greater in 2011 than in 2010. In addition, the N rate effect on RLD was also affected by grafting in 2010 (Tables 3 and 4). Except for the N rate at 112 $kg \cdot ha^{-1}$, grafting with the two rootstocks significantly increased the RLD of 'Florida 47' plants at each of the two higher N rates (i.e., 224 and 336 $kg \cdot ha^{-1}$ N) by 58 and 118%, respectively (Table 4).

The RLD significantly decreased with soil depth during both seasons (Fig. 2). The

Table 2. Effects of the nitrogen (N) fertilization rate and grafting with 'Multifort' and 'Beaufort' rootstocks on tissue N concentrations of 'Florida 47' tomato plants during the 2010 and 2011 field trials in Live Oak, FL.

Treatment	N concn (mg·g^{-1} DW)			
	Leaf blade	Petiole	Stem	Fruit
		2010		
N rate (kg·ha^{-1})				
56	24.35 c	19.78	16.96 b	26.05 c
112	28.43 bc	18.46	17.89 ab	26.15 c
168	28.52 bc	18.08	16.68 b	27.76 ab
224	32.55 ab	20.50	18.44 ab	27.87 ab
280	31.75 ab	17.98	19.56 a	26.46 bc
336	33.97 a	22.52	20.15 a	28.04 a
Contrast[z]	L***		L**	L*
Graft[y]				
FL/BE	30.87	17.82 b	18.46	27.01
FL/MU	30.74	21.39 a	19.11	26.92
FL	28.18	19.46 a	17.27	27.23
		2011		
N rate (kg·ha^{-1})				
56	27.75 b	12.90	12.62 c	23.60
112	29.40 ab	13.58	13.19 bc	24.24
168	31.40 ab	13.66	13.99 bc	23.72
224	32.77 ab	14.18	13.75 bc	23.54
280	32.73 ab	14.36	14.70 ab	25.10
336	34.46 a	14.03	16.20 a	24.66
Contrast[z]	L***		L***	
Graft[y]				
FL/BE	32.03	13.60	14.17	23.92
FL/MU	32.22	14.37	13.98	24.31
FL	30.01	13.38	14.07	24.20

[z]Linear (L), quadratic (Q), and cubic (C) polynomial contrasts were performed; *,**,*** significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[y]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL = nongrafted 'Florida 47'.
Means within a column followed by the same letters do not differ significantly at $P \leq 0.05$ according to Tukey's test.

Table 3. Analysis of variance of the effects of the N fertilization rate, grafting with 'Multifort' and 'Beaufort' rootstocks, root sampling position, and soil depth on root length density and root surface area density of 'Florida 47' tomato plants during the 2010 and 2011 field trials in Live Oak, FL.

Effect	*P* for RLD		*P* for RSAD	
	2010	2011	2010	2011
Main effect				
N rate (N)	<0.01	<0.001	0.077	0.781
Graft (G)	<0.0001	0.001	0.002	0.004
Position (P)	<0.0001	<0.0001	<0.0001	<0.0001
Depth (D)	<0.0001	<0.0001	<0.0001	<0.0001
Two-way interaction				
N×G	0.025	0.359	0.374	0.545
N×P	0.985	0.909	0.556	0.132
N×D	0.189	0.036	0.128	0.982
G×P	0.204	0.781	0.600	0.603
G×D	0.014	0.035	0.105	0.115
P×D	<0.0001	<0.0001	<0.0001	<0.0001
Three-way interaction				
N×G×P	0.304	0.779	0.814	0.174
N×G×D	0.272	0.446	0.435	0.405
N×P×D	0.910	0.744	0.975	0.527
G×P×D	0.733	0.613	0.589	0.767
Four-way interaction				
N×G×P×D	0.669	0.909	0.907	0.535

RLD = root length density; RSAD = root surface area density.

majority of the root system was present in the upper soil layers. Approximately 60% to 62% of the root system was concentrated in the uppermost 15 cm in contrast to 5% to 6% found in the soil layer at 60 to 90 cm. The significant N rate × soil depth interaction in 2011 showed that the increase in the RLD from 112 to 224 kg·ha^{-1} N was primarily limited to the top 15 cm of soil (Table 4). Such an interaction was not observed in 2010.

In addition, a significant interaction was observed between RLD responses in the layers of soil and the effects of grafting during both seasons (Tables 3 and 5). The root systems of all grafted and nongrafted plants were concentrated in the uppermost 15 cm of soil, where ≈58% to 66% of the total detected root length was found. (Table 5). However, the RLD in this layer was increased by ≈74% and 64% in 2010 and 2011, respectively, for grafted plants (Table 5). When deeper soil was examined, the RLD values were similar for grafted and nongrafted plants, except the grafted plants showed a higher RLD in the 60- to 90-cm layer in 2010, and FL/MU had a higher RLD than FL in the 30- to 60-cm layer in 2011 (Table 5).

In addition to variations with the soil depth, the RLD also varied with horizontal distance from the plant stem. Values for RLD measured at the plant base (P1) were significantly higher than those 15 cm away (P2), with the exception of similarities observed in the deeper levels (60–90 cm) in 2011 (Table 5). On average, the RLD at P1 was greater than that at P2 by ≈79%, and the reduction in the RLD from P1 to P2 was typically more pronounced at the top of the soil profile (0–15 cm).

Root surface area density. The RSAD did not differ among the three N rates, but significant effects were evident for grafting, soil depth, and horizontal distance from the stem during both seasons (Table 3). More specifically, RSAD measurements on FL/BE and FL/MU were ≈64% greater than that of FL during both seasons (Fig. 3). The RSAD also decreased with soil depth during both seasons, with ≈65% of the total RSAD in the top 15 cm of soil (Fig. 2).

Discussion

Rootstock and N rate effects on plant growth and NUE. The N nutrition influences leaf growth, leaf area duration, and photosynthetic rate (per unit leaf area), all of which impact the production of assimilates for plant growth (Below, 2002). In the present study, the LAI and aboveground biomass generally increased as N rates increased from 56 to 224 kg·ha^{-1}. These responses were likely the result of accelerated growth rates attributable to N-based increases in carbon assimilation and net primary productivity. These effects of N fertilization rates on tomato growth are consistent with those found by previous research of nongrafted plants (Elia and Conversa, 2012).

In the present work, the LAI and dry matter accumulation of 'Florida 47' tomato grafted onto two interspecific tomato hybrid rootstocks were significantly greater than those of nongrafted 'Florida 47' controls. Such differences reflected the potential of vigorous rootstocks to enhance the growth of grafted plants. The results are consistent with those of previous reports of grafted tomato (Di Gioia et al., 2010; Sánchez-Rodríguez et al., 2014; Suchoff et al., 2018a). Neocleous (2015) reported greater dry biomass for shoots of grafted than of nongrafted melon plants and suggested the involvement of increased photosynthetic capacity associated with biochemical functions at the chloroplast

Table 4. Interaction effects between the nitrogen (N) fertilization rate and grafting with 'Multifort' and 'Beaufort' rootstocks in 2010 and between the N rate and soil depth in 2011 on root length density during the field trials in Live Oak, FL.

	Root length density ($cm \cdot cm^{-3}$)						
	Graft[z] 2010			Soil depth (cm) 2011			
N rate ($kg \cdot ha^{-1}$)	FL/BE	FL/MU	FL	0–15	15–30	30–60	60–90
112	0.43 Ab	0.48 Ab	0.40 Aa	0.99 Ab	0.41 Ba	0.14 Ca	0.11 Ca
224	0.57 Ab	0.60 Ab	0.37 Ba	1.81 Aa	0.65 Ba	0.20 Ca	0.15 Ca
336	0.65 Aa	0.79 Aa	0.33 Ba	1.43 Aab	0.49 Ba	0.15 Ca	0.13 Ca

[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL = nongrafted 'Florida 47'.
Means within a row followed by the same uppercase letters and means within a column followed by the same lowercase letters do not differ significantly at $P \leq 0.05$ according to Tukey's test.

Table 5. Interaction effects between grafting with 'Multifort' and 'Beaufort' rootstocks and soil depth and between the sampling position and soil depth on root length density during the 2010 and 2011 field trials in Live Oak, FL.

	Root length density ($cm \cdot cm^{-3}$)				
	Graft[z]			Position[y]	
Soil depth (cm)	FL/BE	FL/MU	FL	P1	P2
			2010		
0–15	1.40 Aa	1.56 Aa	0.85 Ba	1.70 Aa	0.82 Ba
15–30	0.47 Ab	0.52 Ab	0.37 Ab	0.60 Ab	0.33 Bb
30–60	0.19 Ac	0.27 Ac	0.16 Ac	0.28 Ac	0.15 Bc
60–90	0.13 Ac	0.15 Ad	0.08 Bc	0.15 Ac	0.09 Bc
			2011		
0–15	1.58 Aa	1.66 Aa	0.99 Ba	1.92 Aa	0.90 Ba
15–30	0.54 Ab	0.62 Ab	0.38 Ab	0.65 Ab	0.38 Bb
30–60	0.17 ABc	0.21 Ac	0.11 Bc	0.20 Ac	0.12 Bc
60–90	0.12 Ac	0.15 Ac	0.12 Ac	0.15 Ac	0.11 Ac

[z]FL/BE = 'Florida 47' grafted onto 'Beaufort'; FL/MU = 'Florida 47' grafted onto 'Multifort'; FL = nongrafted 'Florida 47'.
[y]P1 = at the plant base; P2 = at 15 cm from the tomato plant.
Means within a row followed by the same uppercase letters and means within a column followed by the same lowercase letters do not differ significantly at $P \leq 0.05$ according to Tukey's test.

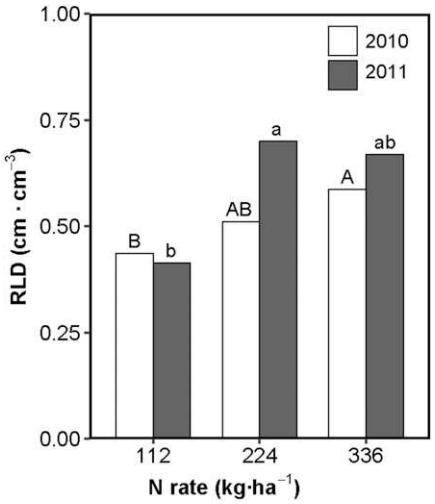

Fig. 1. Effects of the nitrogen (N) fertilization rate on root length density (RLD) during the 2010 and 2011 field trials in Live Oak, FL. Bars with the same uppercase letters (2010) or lowercase letters (2011) do not differ significantly at $P \leq 0.05$ within each season.

level. In the present study, enhanced growth of grafted plants also led to a greater accumulation of N in aboveground tissues than in nongrafted controls. However, this enhanced N accumulation was related more to the greater biomass of grafted plants than to increases in N concentrations of the plant tissues. This result is similar to our previous findings of grafted tomato plants under greenhouse conditions (Djidonou et al., 2017). In contrast, Leonardi and Giuffrida (2006) reported significant differences between N concentrations in tomato plants grafted onto 'Beaufort' and those in self-grafted control plants.

NUpE is a physiological parameter that can be used to evaluate the effectiveness of fertilizer N recovery relative to N uptake by the plant. An analysis of NUpE, defined here as the percentage of N recovered in the aboveground total biomass relative to the applied N, showed a decreasing trend in response to an increasing N fertilization rate. This response is in accordance with that of previous studies of tomato production (Elia and Conversa, 2012; Singandhupe et al., 2003; Zotarelli et al., 2009b). More specifically, Zotarelli et al. (2009b) estimated NUpE ranging from 27% to 73% over a 3-year study involving N rates of 330, 220, and 176 $kg \cdot ha^{-1}$, whereas Scholberg et al. (2000) estimated the values of NUpE to be 36% to 71% for the N rates ranging from 333 to 133 $kg \cdot ha^{-1}$ in drip-irrigated tomato plants grown in north Florida. The general trend of the NUpE to decrease as N rates increase is consistent with the observation that incremental increases in N application fail to generate consistent increases in harvestable yields (Cabello et al., 2009). Moreover, with a limited supply of available N in the growing media, the nutrient demand for growth maintenance could be met by enhanced absorption of mineralized soil N (Jamil-Mohammad, 2004). For instance, Sweeney et al. (1987) reported that in sandy soils of Florida, 2 to 4% of the organic soil N could become available for plant uptake during the growing season; as a result, the N supply from mineralization could be ≈10 to 40 $kg \cdot ha^{-1}$ N during the growing season. Hence, effects of the N fertilization rate could be confounded by contributions from soil N mineralization. Overall, grafted plants, especially those with 'Multifort' rootstock, showed significantly greater NUpE and NUE than nongrafted plants. Particularly, in 2011, an 18% greater NUpE was observed for plants grafted onto 'Multifort' compared to 'Beaufort', indicating that 'Multifort' might be more efficient at N uptake. Similar improvements in NUE have also been observed for melons when grafted with selected rootstocks (Colla et al., 2010; Colla et al., 2011). Although more in-depth research is still needed to fully understand the contributing factors, a major role has been implicated for the increased nitrate reductase activity involved in the NUE improvement of some grafted cucurbit crops (Nawaz et al., 2017). The increased NUE could also be due to the enhanced growth and sink demand of grafted plants relative to nongrafted controls. Recently, Albornoz et al. (2018) reported the increased expression of root plasma–membrane nitrate transporters LeNRT1.1 and LeNRT1.2 when tomato plants were grafted with a vigorous rootstock. In contrast, only the LeNRT1.2 was expressed in nongrafted plants. The authors suggested that this difference in gene expression could have contributed to the enhanced nitrate uptake and use in grafted tomato plants. Similarly, working with a population of recombinant inbred lines from *S. pimpinellifolium*, Asins et al. (2017) found that selected rootstock genotypes can enhance NUpE in tomato. Based on an analysis of rootstock quantitative trait loci that mediate the leaf N concentration, these authors also demonstrated a genetic relationship between the rootstock hormone (especially cytokinin trans-Zeatin and salicylic acid) and NUpE.

Influence of grafting with vigorous rootstocks on root system architecture. The more extensive and vigorous root system of grafted vegetable plants is often credited with a major role in the observed crop vigor and yield enhancement of grafted plants (Martínez-Ballesta et al., 2010; Oztekin et al., 2009). However, research regarding the physical characteristics of grafted plant root systems, especially under field production conditions, is scarce. During a comparison of root traits of several tomato rootstocks under greenhouse conditions, Suchoff et al. (2017) found significant differences among the rootstocks

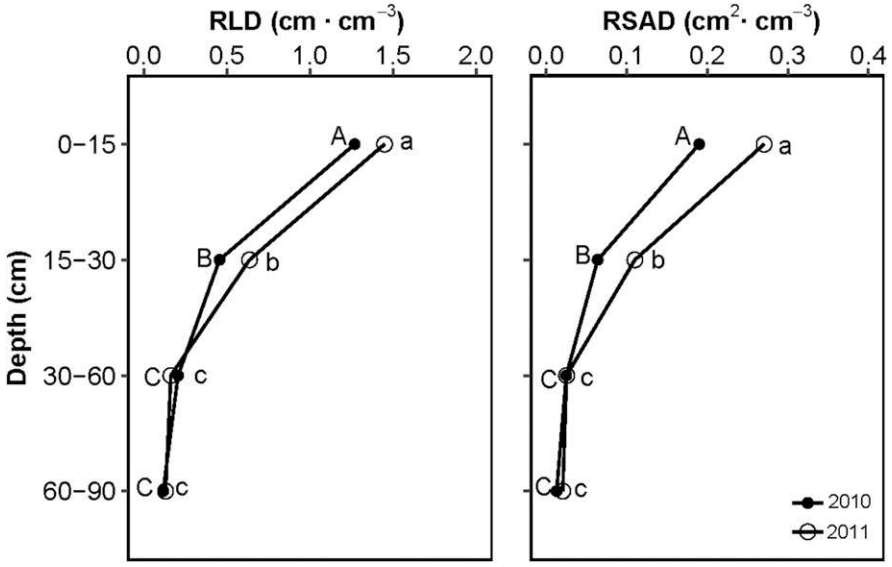

Fig. 2. Root length density (RLD) and root surface area density (RSAD) at different soil depths during the 2010 and 2011 field trials in Live Oak, FL. Values with the same uppercase letters (2010) or lowercase letters (2011) do not differ significantly at $P \leq 0.05$ within each season.

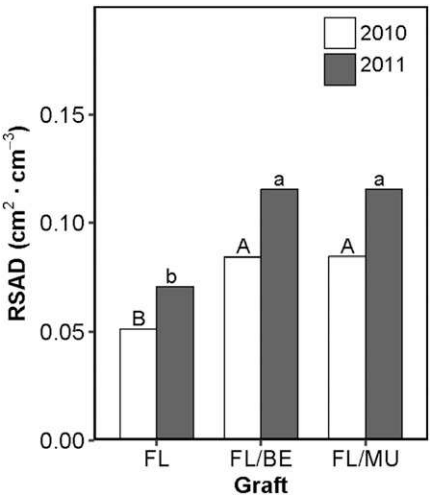

Fig. 3. Effects of grafting with 'Multifort' and 'Beaufort' rootstocks on root surface area density (RSAD) during the 2010 and 2011 field trials in Live Oak, FL. Bars with the same uppercase letters (2010) or lowercase letters (2011) do not differ significantly at $P \leq 0.05$ within each season.

evaluated, with the 'RST-04–106-T' rootstock exhibiting the greatest total root length. Similar enhancements of the root surface area were also reported by Kakita et al. (2015) for grafted tomato. Moreover, Huang et al. (2016) found higher root volumes and root surface areas in grafted watermelon relative to nongrafted watermelon. Modifications of root characteristics were suggested to improve water and nutrient uptake, which would increase the growth of the scion. In the present study, we compared the RLD and RSAD of grafted tomato plants to those of nongrafted plants at different soil depths, distances from the stem, and in response to varying N rates. In general, the capacity of a root system to explore the soil profile can be measured by the values of RLD and RSAD (Munoz-Arboleda et al., 2006). Regardless of grafting, the RLD was more concentrated within the 0- to 15-cm soil layer. This root distribution is consistent with those reported by previous studies of tomato and melon plants (Lecompte et al., 2008; Miller et al., 2013; Zotarelli et al., 2009a). The concentration of roots in the upper layer of soils is closely related to the frequent applications of nutrients and water, which stimulate root proliferation and growth (Jackson and Bloom, 1990). Consistent with previous reports, a decreasing trend in RLD according to the soil profile was also noted in our study. The limited distribution of roots in the deeper soil profile was largely due to the greater soil bulk density and increased level of mechanical resistance (Zotarelli et al., 2009a).

The rootstock-modulated effect on RLD varied with soil depth in the present study. Although the RLD was similar for grafted and nongrafted plants at soil depths between 15 and 30 cm, differences were evident in the uppermost 15 cm. In this layer, the RLD values were significantly greater for grafted plants during both seasons. Some differences also appeared in deeper layers; grafting with 'Multifort' led to a greater RLD at 30 to 60 cm in 2011, and at 60 to 90 cm in 2010. The increase of the RLD due to grafting, especially in the uppermost soil layer, may have positively influenced nutrient uptake potential and may have led to the enhancement of aboveground N accumulation and NUE in grafted tomato plants observed during this study. In addition, the potential for increased RLD in grafted plants was evident in the case of the 'Multifort' rootstock at soil depths more than 30 cm. The more extensive root system of such rootstocks would allow greater access to nutrients, especially N, that can easily move beyond the active root zone. However, further studies are still warranted to determine how these improved root traits are related to nutrient uptake and movement, especially nitrate, in the soil profile. Such findings can be even more meaningful for sandy soils with poor capacities to hold water and nutrients.

In general, the RLD increased as the N rate increased from 112 to 224 kg·ha^{-1} during both seasons. Interestingly, grafting appeared to be the primary driver of RLD responses to N rates in 2010, when nongrafted plants showed minimal effects of N rates on the RLD. The greatest RLD values were reached by grafted plants at 336 kg·ha^{-1} N. As N application rates increased, the number of tomato roots per unit area of soil profile increased (Sainju et al., 2001), indicating significantly greater values at 90 and 180 kg·ha^{-1} N compared with 0 kg·ha^{-1} N during one of the two seasons. In contrast, work by Jackson and Bloom (1990) showed no clear relationship between tomato root distribution and soil N availability. Root architecture often changes in response to availability and distribution of inorganic nutrients in the soil (Lopez-Bucio et al., 2003). High levels of N fertilization also tend to promote shoot growth at the expense of root development. As a result, the shoot:root ratio typically decreases in most plant species when N availability is reduced (Ågren and Franklin 2003).

Grafting with selected rootstocks as an effective horticultural practice to promote enhancement in growth and yield traits of a range of vegetable crops including tomato beyond disease management deserves more in-depth research. The grafting benefits are likely attributed to the rootstock-mediated influence of several physiological and biochemical processes, including the increased activity of enzymes responsible for nutrient assimilation, especially nitrate reductase activity (Pulgar et al., 2000; Ruiz and Romero, 1999), and changes in endogenous hormone balance, especially auxin and cytokinin (Aloni et al., 2010; Lee and Oda 2003). In addition, the long-distance transport of molecules such as proteins and RNAs from the rootstock might positively affect the shoot growth (Albacete et al., 2015; Venema et al., 2017).

Conclusions

In the present study, plant growth, N accumulation, NUE, and NUpE were improved by grafting regardless of the N fertilization rate when 'Florida 47' tomato plants were grafted onto either of two vigorous and interspecific hybrid rootstocks ('Beaufort' and 'Multifort'). Grafting with the interspecific hybrid rootstock improved RLD and RSAD, particularly within the uppermost 15 cm of soil. This could contribute directly to the enhanced NUpE of grafted plants. Additional research will aid in the delineation of genetic variations among tomato rootstocks in terms of the capacity to enhance root architecture and, thus, nutrient acquisition of grafted plants. Grafted transplants may be promising for the reduction of N leaching while enhancing N uptake and fertilizer use efficiency during tomato production.

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