

# Rootstock Effect on Grafted Tomato Transplant Shoot and Root Responses to Drying Soils

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**Abstract.** Improvement of crop water use is imperative. Plants' responses to limited water can dictate their ability to better use available resources and avoid prolonged and severe stress. The following study was conducted to determine how tomato (*Solanum lycopersicum*) rootstocks with different root system morphologies respond to drying soils. Plants were grown in pots containing an inorganic substrate composed of calcined clay and sand in a greenhouse on North Carolina State University's campus. The heirloom tomato cultivar Cherokee Purple was used as the scion for 'Beaufort' and 'Shield' rootstocks as well as the self-grafted control. These rootstocks were assigned either normal or reduced irrigation treatments. Plants grown under the normal irrigation schedule were weighed and watered daily to maintain container capacity for one week. Those receiving reduced irrigation had all water withheld for one week, at which point strong midday wilting became evident. Shoot physiological and morphological data as well as root morphological data were collected at the end of the study. A constitutive positive increase on relative water content, leaf area, stomatal conductance ( $g_s$ ), and net  $CO_2$  assimilation rate was observed with scions grafted on 'Beaufort'. In addition, this rootstock had a significantly longer total root system (118.6 m) compared with 'Shield' (94.9 m) and the self-grafted control (104.2 m). Furthermore, 76.4% of the total root length observed in 'Beaufort' was composed of very thin diameter roots ( $\emptyset < 0.5$  mm), which was higher than 'Shield' (73.67%) and the self-grafted control (69.07%). The only significant rootstock  $\times$  irrigation interaction observed was for effective quantum yield of photosystem II ( $\Phi_{PSII}$ ). At normal irrigation there were no differences among the rootstock treatments; however, at reduced irrigation 'Beaufort' had significantly higher  $\Phi_{PSII}$  than both 'Shield' and the self-grafted control. These results may explain some of the improved production and water use efficiency observed in field trials using 'Beaufort' rootstock, and data secured may allow for better screening of rootstocks for improved water use efficiency in the future.

Drought poses the greatest threat to global food production. Current agricultural practices use  $\approx 70\%$  of the available water; to meet the demands of a growing global population, water consumption will need to increase by 70% to 90% by 2050 (Molden, 2007; Somerville and Briscoe, 2001). This untenable demand for water is further exacerbated

by the predicted increase in severity of erratic weather and drought due to climate change (Trenberth, 2011). As such, it is of utmost importance that agricultural production systems improve crop resilience and efficiencies in water resource utilization.

A plant's ability to respond and adapt to drying soils directly impacts its ability to withstand brief dry periods and also avoid long-term drought stress. Plants have evolved intricate molecular, biochemical, and morphological responses to water stress (Bray, 1997; Hsiao, 1973; Shao et al., 2008). These responses can be grouped into either dehydration tolerance and/or dehydration avoidance based on their physiological impacts under drought conditions (Blum, 2005; Levitt,

1972). Dehydration tolerance refers to adaptations that allow a plant to maintain function even in a dehydrated state (Levitt, 1972). Examples of these are relatively rare and exotic and include such mechanisms as seed embryo dehydration and the dehydration tolerance seen in the resurrection plant (*Crotonostigma plantagineum*). Developing more resilient food crops that use dehydration tolerance mechanisms is difficult due to the rarity of these traits among species (Blum, 2005). Dehydration avoidance is defined as the ability to maintain water status under limited water conditions (Levitt, 1972). These traits include early flowering, reduced leaf area, stomatal closure, increased root:shoot ratio, alteration of root morphology and architecture, and osmotic adjustments (Blum, 2005).

Full or partial stomatal closure is one of the earliest drought avoidance responses to water stress and limits transpirational water loss; however, this reduction in  $g_s$  leads to a concomitant reduction in  $CO_2$  diffusion and consequent reduction in photosynthesis and carbon assimilation (Chaves, 1991; Hsiao, 1973). As such, developing more drought tolerant crops based on stomatal traits may improve overall water use, but it can lead to yield reductions even under well-watered conditions (Deikman et al., 2012).

As the site of water uptake and plant-soil interface, root systems have been the focus of substantial drought stress research. Numerous root system phenotypes based on depth, spatial distribution, and diameter have been shown to improve water acquisition under limiting conditions (Comas et al., 2013; Ho et al., 2005; Huang and Eissenstat, 2000; Mickelbart et al., 2015). Unfortunately, breeding for specific root system phenotypes while maintaining elite fruit traits is exceedingly difficult (Malamy, 2005; Wasson et al., 2012). One potential means to selecting both fruit and root traits is through grafting.

Essentially a root transplant, grafting offers the ability to manage numerous soil-borne pathogens that affect solanaceous and cucurbitaceous crops (Louws et al., 2010). Furthermore, recent work has demonstrated the ability of rootstocks to improve fruit quality, soil resource use efficiency, as well as combined biotic and abiotic stress tolerance (Kyriacou et al., 2017; Roupael et al., 2018). Certain rootstocks have demonstrated the ability to improve water use efficiency in susceptible scions (Djidonou et al., 2013; Schwarz et al., 2010). In their open-field grafted tomato study, Djidonou et al. (2013) showed that rootstocks can increase irrigation water use efficiency regardless of water regime applied. This response indicates a constitutive rootstock effect on growth and yield. Many of the commercially available rootstocks, including those shown to improve water use efficiency, have significantly different root system morphologies (Suchoff et al., 2017). Whether these rootstocks respond differently to drying soils at a root system morphological level is unknown. As such, the objectives of the following study

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were to 1) compare root systems of two commercially available tomato rootstocks with different root system morphology when reducing available water; 2) determine if root system morphology in tomato rootstocks changes with available water; and 3) compare rootstock effects on scion morphology and physiology with reduced available water.

### Materials and Methods

Studies were conducted in an air-inflated double-layer polyethylene greenhouse at North Carolina State University's Horticultural Field Laboratory during the months of July and August 2017. Temperatures were maintained ( $30 \pm 3$  °C day/ $25 \pm 3$  °C night) with evaporative cooling (Kool-Cel® Aluminum PDR system, 10.2 cm cellulose pad; Acme Engineering & Manufacturing Corp., Mukogee, OK).

**Transplant preparation.** The heirloom tomato cultivar Cherokee Purple was self-grafted as the control (C) and scion on 'Beaufort' (B; De Ruiter, St. Louis, MO) and 'Shield' (S; Rijk Zwaan, Salinas, CA) rootstocks. These two rootstocks were chosen due to their differing root system morphologies (Suchoff et al., 2017) and irrigation water use efficiency (Suchoff et al., 2018). All plants were started in 72-cell plug trays (T.O. Plastics, Clearwater, MN) filled with a mixture of calcined clay (Turface MVP; Profile Products LLC, Buffalo Grove, IL) and sand (#20 Pool Filter Sand; Aquabrite®, Pleasanton, CA; 2:1 v/v). This mixture allows for thorough extraction and cleaning of roots while still maintaining physical properties similar to field soils (Manavalan et al., 2010; Suchoff et al., 2017). Because of the slow germination of B, it was seeded three days before S and C. When seedlings developed two or three true leaves and had a hypocotyl diameter of 2.0 mm they were grafted using the Japanese tube-graft method (Rivard and Louws, 2006). A 45° angle cut was made on both the rootstock and scion below the cotyledon. Scion and rootstock were held together using a 2.0 mm diameter silicone graft clip (Johnny's Selected Seeds, Fairfield, ME). Grafts were healed in transparent plastic storage bins (67.3 cm length  $\times$  40.6 cm height  $\times$  31.8 cm width; Steri-lite®, Townsend, MA) where they received  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of photosynthetically active radiation ( $4 \times 6500$  °K Spectralux® T5 High Output Fluorescent lamp; Sunlight Supply, Inc., Vancouver, WA) in an indoor, controlled environment maintained at 21 °C. The healing process took 5 d during which the plants were slowly acclimated to reduced relative humidity by gradually opening the top to the storage bin until it was completely off on day 5. Healed plants were moved back to the greenhouse where they were further hardened off for 2 d.

Black polyethylene pots with a volume of 7.33 L and dimensions of 24.1 cm (top diameter)  $\times$  20.3 cm (height)  $\times$  20.3 cm

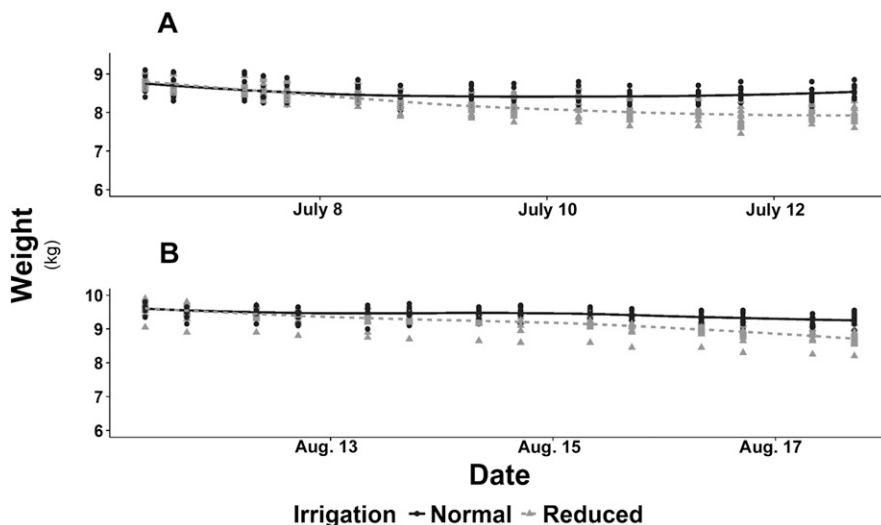


Fig. 1. Pot weight for normal and reduced irrigation treatments during trials one (A) and two (B) fit with a loess curve. Pots were weighed daily at 8:00 AM and 5:00 PM and water added to only the normal irrigation treatment pots to maintain pot weight at container capacity.

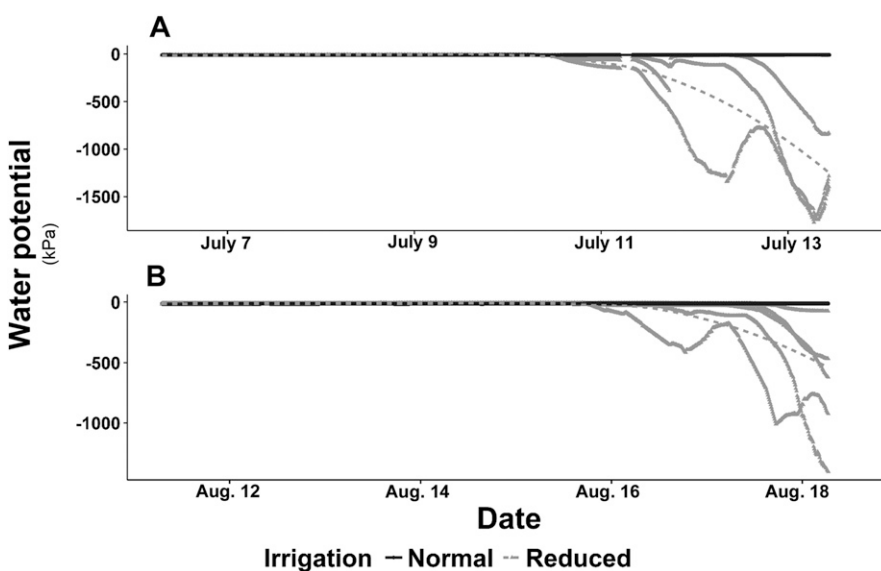


Fig. 2. Water potential for normal and reduced irrigation treatments during trials one (A) and two (B) fit with a loess curve. Readings were taken using water potential sensors placed in one pot per irrigation treatment within a block ( $n = 5$  sensors per irrigation treatment per trial).

Table 1. Results of analysis of variance for the impact of rootstock and irrigation on greenhouse grown tomato scion morphology and physiology.

Effect	df	Leaf dry wt	Stem dry wt	Shoot <sup>2</sup> dry wt	Leaf RWC <sup>y</sup>	Stem RWC	Leaf area	Specific leaf area <sup>x</sup>	$g_s^w$	$A^v$	$\Phi_{PSII}^u$
Rootstock	2	NS	NS	NS	***	NS	***	**	*	**	***
Irrigation	1	***	***	***	***	***	***	***	***	***	***
Rootstock $\times$ Irrigation	2	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

NS, \*, \*\*, \*\*\* denote nonsignificant at  $P \leq 0.05$  or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>2</sup>Shoot dry weight is the combination of leaf and stem dry weights.

<sup>y</sup>Relative water content.

<sup>x</sup>Calculated as leaf area divided by leaf dry weight.

<sup>w</sup>Stomatal conductance.

<sup>v</sup>Net CO<sub>2</sub> assimilation rate.

<sup>u</sup>Efficiency of photosystem II, calculated as the proportion of photons used by photosystem II for photochemistry.

(bottom diameter) (Poly-Tainer #3 Short; Hummert International, Earth City, MO) were lined with woven 20  $\times$  20 mesh of

0.02 cm diameter thread ( $\approx 0.016$  cm<sup>2</sup> opening size; Clear Advantage Charcoal Fiber Glass Insect Screen; New York Wire,

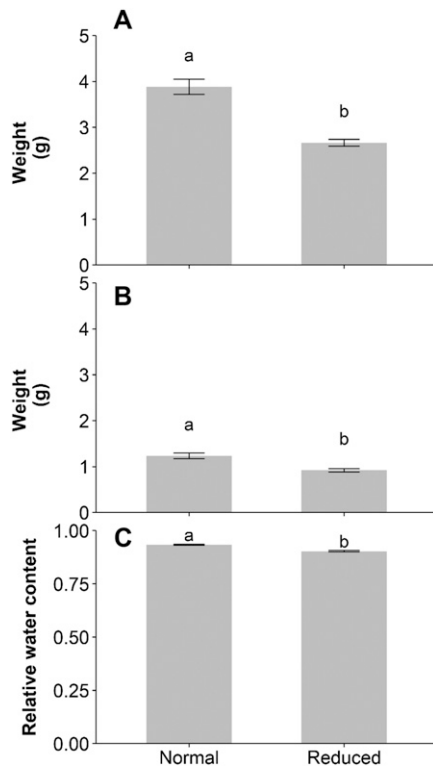


Fig. 3. Main effect of irrigation on leaf dry weight (A), stem dry weight (B), and stem relative water content (C)  $\pm$ SE. Means with common letters within a response are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five blocks, and three rootstock treatments (n = 30 data points for each mean).

Hanover, PA). Lined pots were filled with 7 L of the calcined clay/sand mixture, and 63 g of controlled-release fertilizer (Osmocote® Plus 15–9–12; Everris, Geldermalsen, Netherlands) was thoroughly mixed throughout the media. Pots were weighed, watered thoroughly, covered with aluminum foil to prevent evaporation, and then placed in 11 cm of standing water for 24 h. This soaking period allowed for complete saturation of the media. The pots were removed from the water after 24 h and allowed to drain freely. Once free-drainage from the bottom of the pots stopped ( $\approx 15$  min) they were weighed to determine individual container capacity values (White and Mastalerz, 1966).

**Experimental setup.** The healed plants were transplanted into the pots at container capacity, the aluminum foil cover placed back on the pot, and re-weighed to account for the additional weight of the transplant root ball. Individual pots were designated an irrigation treatment (normal or reduced) and arranged in a full factorial (3 rootstocks  $\times$  2 irrigation treatments) randomized complete block design with five blocks (n = 30 pots). Blocks were arranged along the length of a greenhouse bench to account for any temperature gradient moving away from the evaporative cooling pad. All treatments were watered daily to allow for proper root establishment for the first five days. Irrigation treatments were applied following this

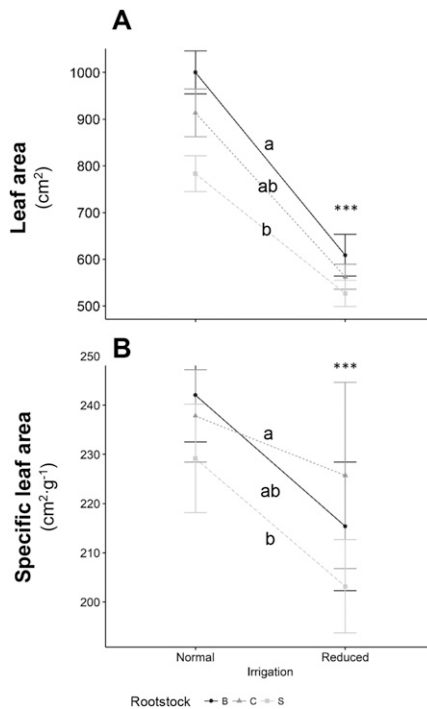


Fig. 4. Main effect of rootstock and irrigation on total leaf area (A) and specific leaf area (B)  $\pm$ SE. Means with common letters within a response are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five blocks, and two irrigation treatments (n = 20 data points for each mean). Rootstock treatments include self-grafted 'Cherokee Purple' tomato (C), 'Cherokee Purple' grafted onto 'Beaufort' rootstock (B), and 'Cherokee Purple' grafted onto 'Shield' (S) rootstock. \*\*\* denotes a significant difference between irrigation treatment means (Tukey's HSD;  $\alpha = 0.05$ ) which represent the average of two trials, five blocks, and three rootstock treatments (n = 30 data points for each mean).

five-day period. All pots were weighed at 8 AM and the volume of water lost due to transpiration was determined by subtracting this morning weight from the container capacity weight and added back to those containers receiving the normal irrigation treatment to maintain the containers' close-to-container capacity. For those pots receiving the reduced irrigation treatment, all water was withheld for 7 d, at which point strong midday wilting was evident and the study was ended. The first trial of the study started on 6 July 2017 and ended 13 July, and then repeated from 11 Aug. to 18 Aug. 2017.

**Data collection.** Before transplanting, matric water potential sensors (MPS-6; METER Group, Pullman, WA) were placed in the middle of two pots per block, one pot receiving the reduced treatment, and the other receiving the normal irrigation treatment. Soil matric water potential readings were taken every hour and stored in a data logger (EM50; METER Group). Pot weight was recorded at 8:00 AM and 5:00 PM.

Leaf gas exchange and fluorescence measurements were taken the day before termination of each trial. Measurements were

taken on the terminal leaflet of the two most recently matured, fully expanded leaves using an open gas exchange system coupled with a leaf chamber fluorometer (LI-6400XT; LI-COR, Inc., Lincoln, NE). Net CO<sub>2</sub> assimilation ( $A$ ,  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ),  $g_s$  ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), and effective quantum yield of photosystem II ( $\Phi_{\text{PSII}}$ ) were measured between 10 AM and 1 PM. Photosynthetic photon flux density within the chamber was set to  $1400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , with temperature and relative humidity maintained at levels matching those inside the greenhouse. The sensor head was placed on each leaflet and left for two to three minutes until values of  $A$  and  $g_s$  stabilized at which point the measurement was taken.

Leaf and root tissue were collected upon termination of the experiment. Leaf and stem tissue were partitioned for fresh weight measurements. Following weighing, leaf area was measured using a leaf area meter (LI-3100C area meter; LI-COR, Inc.). Stem and leaf tissue were then dried for 72 h at 70 °C for dry weight measurements. These data were used to determine relative water content and specific leaf area with the following formulas:

$$\text{Relative water content} = (\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight} \quad [1]$$

$$\text{Specific leaf area} = \text{Leaf area} / \text{Leaf dry weight} \quad [2]$$

Eq. [1] was used to calculate relative water content of leaf and stem tissue, separately.

Root balls were excavated from the media and any roots that broke off during this process were carefully collected following the protocols of Suchoff et al. (2017). Roots were rinsed thoroughly to remove any attached media and placed in a 0.5 g·L<sup>-1</sup> neutral red dye solution (Sigma Aldrich Co., St. Louis, MO) for 24 h at 6.7 °C. The dyeing process improved resolution during scanning and image acquisition (Bouma et al., 2000). Roots were rinsed, placed in a 30  $\times$  42 cm acrylic tray filled with 3 cm of water and scanned at 800 dots per inch (dpi) using a flatbed scanner (Epson Expression® 10000XL; Epson America, Long Beach, CA). Resultant images were analyzed with a root system image analysis software (WinRHIZO v. 2012b; Regent Instruments Inc., Quebec, Canada). Data from the image analysis included average root diameter, total root length, and length per diameter class. Three diameter classes in increments of 0.5 mm were used: The first diameter class length (DCL1) represents the total length of all roots with a diameter less than 0.5 mm, DCL2 is the length of roots with diameters between 0.5 mm and 1.0 mm, and DCL3 is the length of those roots with diameters greater than 1.0 mm. Diameter class length data were normalized by dividing by total root length, giving the proportion of root length composed of each diameter class (relative

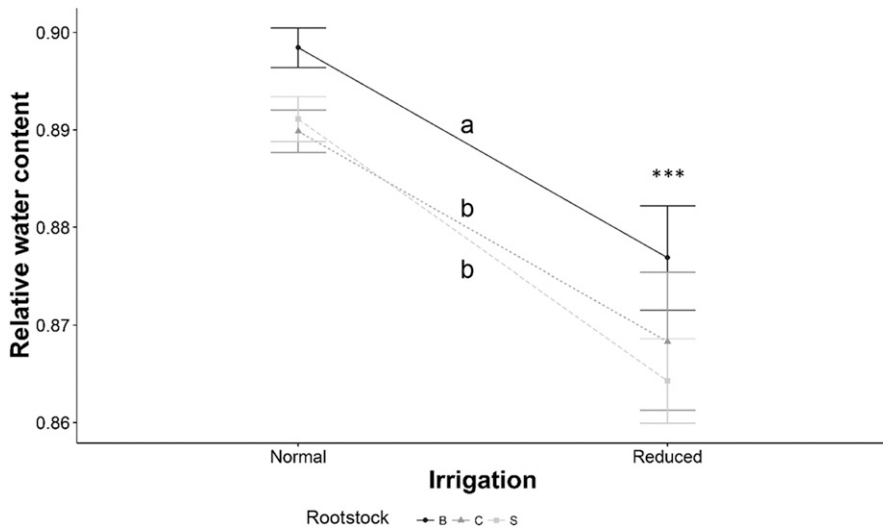


Fig. 5. Main effect of rootstock leaf relative water content  $\pm$ SE. Means with common letters are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five blocks, and two irrigation treatments ( $n = 20$  data points for each mean). Rootstock treatments include self-grafted 'Cherokee Purple' tomato (C), 'Cherokee Purple' grafted onto 'Beaufort' rootstock (B), and 'Cherokee Purple' grafted onto 'Shield' (S) rootstock. \*\*\* denotes a significant difference between irrigation treatment means (Tukey's HSD;  $\alpha = 0.05$ ) which represent the average of two trials, five blocks, and three rootstock treatments ( $n = 30$  data points for each mean).

diameter class length; RDCL). Roots were dried at 70 °C for 24 h (Thelco 130D Laboratory Oven; Precision Scientific Co., Chennai, India) and total root system dry weights collected. Root dry weight data were used to calculate specific root length (total root length/root dry weight) and root:shoot ratio (root dry weight/shoot dry weight).

**Statistical analysis.** Data from the two trials were combined and analyzed using PROC GLIMMIX in SAS (version 9.4; SAS Institute Inc., Cary, NC). Rootstock and irrigation treatments were analyzed as fixed effects with experiment and block nested in experiment as random effects. Proportion data (RDCL,  $\phi_{PSII}$ , relative water contents) were modeled using a beta distribution and canonical link function. Pearson chi-squared statistics divided by the df ( $\phi$ ) were checked for over-dispersion and distribution goodness-of-fit. All other data were assumed normal and checked for heteroscedasticity and outliers. Total root length showed heteroscedasticity, which was ameliorated through a square root transformation of the data. For presentation, total root length data were back-transformed to original scale. The Tukey's honest significant difference post hoc mean separation was conducted for any effect found to be significant ( $P < 0.05$ ).

## Results

Pot weight and water potential were effectively maintained in the normal irrigation treatments through daily watering (Figs. 1 and 2). Withholding water in the reduced irrigation treatments showed a reduction in pot weight and water potential, though water potential among these treatments differed within trials (Figs. 1 and 2).

**Scion morphology and physiology.** The main effect of irrigation affected all scion morphological and physiological responses measured except for  $\phi_{PSII}$  in which the interaction of irrigation and rootstock was significant (Table 1). For those scion morphological and physiological responses affected by irrigation, the reduced irrigation treatment resulted in significantly lower measurements (Figs. 3–6). The effect of rootstock did not affect leaf dry weight but it did affect leaf area and specific leaf area (Table 1). B showed significantly higher leaf area and specific leaf area than S with C intermediate to both (Fig. 4A and B). Leaf relative water content in 'B' was higher than S and C but no difference were observed between the latter two (Fig. 5). This same trend was observed for  $g_s$  and  $A$ ; B had higher levels of both responses compared with S and C with no difference between the latter two (Fig. 6). At normal irrigation,  $\phi_{PSII}$  was similar among the three rootstock treatments; however, at reduced irrigation B had higher  $\phi_{PSII}$  (0.3374) than C (0.2756) and S (0.2670; Fig. 6C).

**Root system morphology.** None of the root system morphological responses were affected by the rootstock  $\times$  irrigation interaction (Table 2). The reduced irrigation treatment resulted in a thickening in average diameter (Table 2; Fig. 7B). This thickening of average root diameter appears to be the result of changes in diameter class length associated with reduced irrigation. The reduced irrigation treatment resulted in a shorter root length for those roots with diameters  $<0.5$  mm (8154.0 cm at normal irrigation and 7380.80 cm at reduced irrigation; Table 3). The thicker roots in DCL2 were unaffected by irrigation treatments (Table 2); however, since total root length decreased with reduced

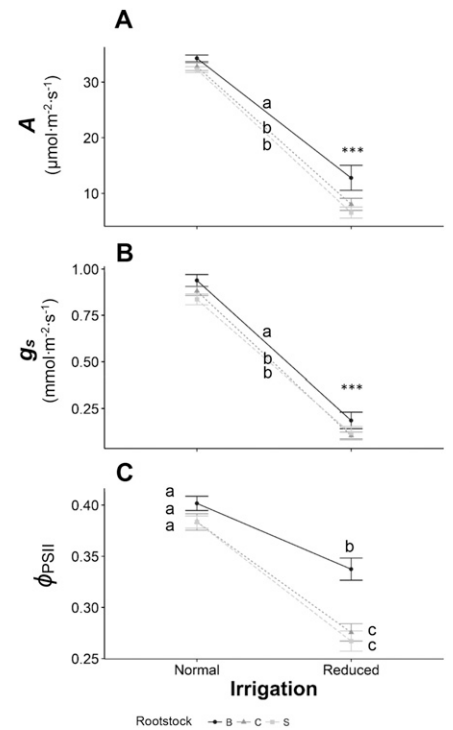


Fig. 6. Main effect of rootstock on net CO<sub>2</sub> assimilation (A), stomatal conductance ( $g_s$ ) (B), and the interaction of rootstock and irrigation on effective quantum yield of photosystem II (C).  $\pm$ SE. Means for net CO<sub>2</sub> assimilation and  $g_s$  with common letters are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five blocks, two irrigation treatments, and two leaf subsamples ( $n = 40$  data points for each mean). Means for effective quantum yield of photosystem II represent the average of two trials, five blocks, and two leaf subsamples ( $n = 20$  data points for each mean). Rootstock treatments include self-grafted 'Cherokee Purple' tomato (C), 'Cherokee Purple' grafted onto 'Beaufort' rootstock (B), and 'Cherokee Purple' grafted onto 'Shield' (S) rootstock. \*\*\* denotes a significant difference between irrigation treatment means (Tukey's HSD;  $\alpha = 0.05$ ) which represent the average of two trials, five blocks, three rootstock treatments, and two leaf subsamples ( $n = 60$  data points for each mean).

irrigation (Fig. 7A) DCL2 roots comprised a larger proportion of the total root length at reduced irrigation (RDCL2 of 0.1832 at full irrigation and 0.2861 at reduced irrigation; Table 3).

Root:shoot ratio was affected by both irrigation and rootstock (Table 2). At reduced irrigation root:shoot ratios increased compared with normal irrigation treatments (Table 4). This increase was due to the concurrent reduction in shoot dry weight and increase in root dry weight (Table 4). Shoot dry weight was unaffected by rootstock treatments; however, root dry weight was significantly higher in C compared with B and S, and thus C had a higher root:shoot ratio.

Total root length and specific root length decreased with reduced irrigation (Fig. 7A and C). Rootstock also affected these two responses: B had the longest total root length followed by C and then S. Specific root length

Table 2. Results of analysis of variance for the impact of rootstock and irrigation on greenhouse grown tomato root systems.

Effect	df	Root dry wt	Root:shoot <sup>z</sup>	Avg root diam	Total root length	Specific root length <sup>y</sup>	DCL1 <sup>x</sup>	DCL2	DCL3	RDCL1 <sup>w</sup>	RDCL2	RDCL3
Rootstock	2	*	**	***	***	***	***	***	***	***	***	**
Irrigation	1	**	***	***	**	***	**	NS	**	**	***	NS
Rootstock × Irrigation	2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, \*, \*\*, \*\*\* denote nonsignificant at  $P \leq 0.05$  or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>z</sup>Ratio of root dry weight to shoot dry weight.

<sup>y</sup>Calculated as total root length divided by root dry weight.

<sup>x</sup>Diameter class length. DCL1 is root length of roots with diameter less than 0.5 mm, DCL2 are roots with diameter between 0.5 and 1.0 mm, and DCL3 are roots with diameter greater than 1.0 mm.

<sup>w</sup>Relative diameter class length. RDCL1 is the proportion of total root length composed of roots with diameter less than 0.5 mm, RDCL2 are roots with diameter between 0.5 and 1.0 mm, and RDCL3 are roots with diameter greater than 1.0 mm.

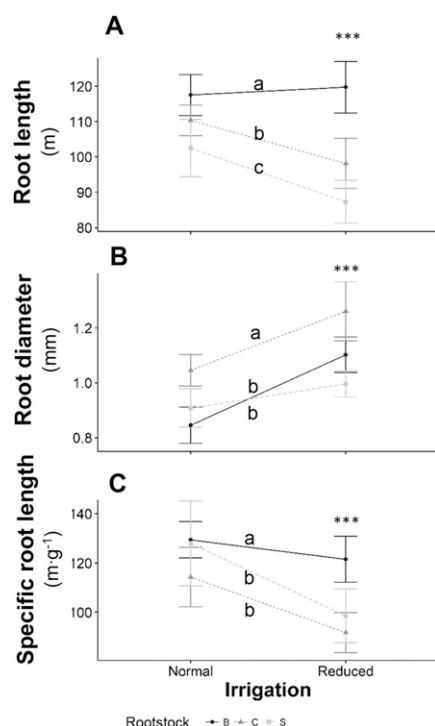


Fig. 7. Main effect of rootstock on total root length (A), average root diameter (B), and specific root length (C)  $\pm$ SE. Means with common letters within a response are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five blocks, and two irrigation treatments ( $n = 20$  data points for each mean). Rootstock treatments include self-grafted 'Cherokee Purple' tomato (C), 'Cherokee Purple' grafted onto 'Beaufort' rootstock (B), and 'Cherokee Purple' grafted onto 'Shield' (S) rootstock. \*\*\* denotes a significant difference between irrigation treatment means (Tukey's HSD;  $\alpha = 0.05$ ) which represent the average of two trials, five blocks, and three rootstock treatments ( $n = 30$  data points for each mean).

was also highest in B and significantly lower in C and S, but no differences were observed between the latter two.

## Discussion

In his April 2000 address at the "South Summit," the then-United Nations Secretary-General Kofi Annan called for an agricultural "Blue Revolution" (The United Nations,

2000). The urgency for improved water use was further voiced in the report to the President on *Agricultural Preparedness and the United States Agriculture Research Enterprise*, which lists improved water use efficiency and resilience in a changing climate as two of the seven major challenges faced by agriculture in the 21st century (PCAST, 2012). Water demands and the length of dry periods are crop- and production-system dependent. In the United States, 91.7% of the total tomato acreage is irrigated and normally does not experience the prolonged drought events that can be encountered in dryland field crops (USDA ERS, 2010). Nevertheless, a concerted effort to improve water use efficiency in vegetable production is imperative to meet current and future demands.

An increasing body of research indicates that grafting can improve water use in herbaceous crops (Kumar et al., 2017). Results from this study demonstrate that using 'Beaufort' as a rootstock can significantly improve relative water content, net  $\text{CO}_2$  assimilation, and  $g_s$  regardless of irrigation regime (Figs. 5 and 6A–B). The absence of significant rootstock  $\times$  irrigation interactions for all responses other than  $\phi_{PSII}$ , and presence of significant rootstock main effects, are indicative of a constitutive, not drought-responsive, rootstock effect (Table 1 and 2). These constitutive rootstock effects were observed by Al-Harbi et al. (2017) and Ibrahim et al. (2014), who both found that the interspecific hybrid tomato rootstock 'Unifort' had a positive effect on vegetative growth (shoot fresh weight, leaf area, leaf dry weight) and yield regardless of irrigation treatments. The significant increase in relative water content we observed when grafting onto 'Beaufort' is in agreement with the findings of Altunlu and Gul (2012). These authors found that under PEG-induced drought stress, 'Beaufort' improved relative water content in the scion, which they partially attribute to the observed increase in the osmoprotectant proline. Osmoprotectants were not investigated in this study; however, many of the differences observed in root system morphologies may explain the increase in water status and photosynthetic activity.

An increase in total root length allows plants to search farther for water, both horizontally and vertically, within the soil profile

(Comas et al., 2013; Mickelbart et al., 2015). Furthermore, a root system composed of thin-diameter, fine roots can improve acquisition of water and overall plant productivity due to an increase in root hydraulic conductance and specific root length (SRL; Comas et al., 2013; Wasson et al., 2012). These root traits appear to be common among herbaceous and woody plants that are adapted to dryer conditions (Hernández et al., 2010; Henry et al., 2012). B showed significantly longer total root length and higher SRL compared with both S and C (Fig. 7A and C). Both B and S had significantly thinner average root diameters compared with C; however, B had a higher percentage (76.40%) of its total root length composed of these thin roots (DCL1; diameter less than 0.5 mm) compared with S (73.67%) and C (69.07%; Table 3). Because B also had a significantly longer total root length, the observed higher RDCL1 amounts to an 1855.1 cm and 2050.5 cm increase in DCL1 roots compared with C and S, respectively (Table 3). Accordingly, the ability of B to maintain higher relative water content, net  $\text{CO}_2$  assimilation, and  $g_s$  may be attributed to its much longer, thinner root system that allows for improved uptake and conductance of water.

The constitutive positive effects of 'Beaufort' were also observed by Djidonou et al. (2013). In their irrigation- and nitrogen-use efficiency field study, the authors found that 'Florida 47' grafted onto 'Beaufort' yielded significantly more than nongrafted 'Florida 47' regardless of irrigation treatments and consequently improved irrigation water use efficiency. Suchoff et al. (2018) showed that, in a high-tunnel, on-farm situation, B had no yield reduction when irrigation was reduced by 50% and produced significantly more than 'Cherokee Purple' grafted onto 'Shield' and nongrafted 'Cherokee Purple'.

Nilsen et al. (2014) conducted a similar study in which they compared rootstock effects on tomato vegetative growth after withholding water. The authors demonstrated that the tomato rootstock cultivar Jjak Kkung reduced the amount of drought stress-induced growth inhibition compared with a nongrafted control or the use of 'Cheong Gang' rootstock. The authors found that 'Jjak Kkung' did not show a significant reduction in leaf area under drought conditions; however, they

Table 3. Effect of rootstock and irrigation on relative diameter class length proportions for greenhouse grown tomatoes.

Rootstock	DCL1 <sup>z</sup> (cm)	DCL2 (cm)	DCL3 (cm)	RDCL1 <sup>y</sup>	RDCL2	RDCL3
'Cherokee Purple' self-grafted	7,214.2 b	2,496.0 a	708.2 a	0.6907 c <sup>x</sup>	0.2399 a	0.0678 a
'Beaufort'	9,069.3 a	2,245.2 b	542.7 b	0.7640 a	0.1893 c	0.0458 c
'Shield'	7,018.8 b	1,954.1 c	511.5 b	0.7367 b	0.2078 b	0.0543 b
Irrigation						
Normal	8,154.0 a	2,229.8	622.9 a	0.7414 a	0.1832 b	0.0552
Reduced	7,380.8 b	2,233.6	551.9 b	0.7214 b	0.2861 a	0.0554

<sup>z</sup>Diameter class length. DCL1 is the length of roots with diameter less than 0.5 mm, DCL2 are roots with diameter between 0.5 and 1.0 mm, and DCL3 are roots with diameter greater than 1.0 mm.

<sup>y</sup>Relative diameter class length. RDCL1 is the proportion of total root length composed of roots with diameter less than 0.5 mm, RDCL2 are roots with diameter between 0.5 and 1.0 mm, and RDCL3 are roots with diameter greater than 1.0 mm.

<sup>x</sup>Means followed by the same letter within DCL or RDCL and main effect are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five replicates and two irrigation treatments for rootstock main effect (n = 20 data points for each mean) and two trials, five replicates, and three rootstock treatments for the irrigation main effect (n = 30 data points for each mean).

Table 4. Effect of rootstock and irrigation on root dry weight, shoot dry weight, and root:shoot ratio for greenhouse grown tomatoes.

Rootstock	Root dry wt (g)	Shoot dry wt <sup>z</sup> (g)	Root:shoot
'Cherokee Purple' self-grafted	1.086 a <sup>y</sup>	4.295	0.258 a
'Beaufort'	0.969 ab	4.648	0.215 b
'Shield'	0.892 b	4.113	0.220 b
Irrigation			
Normal	0.938 b	5.115 a	0.183 b
Reduced	1.027 a	3.589 b	0.286 a

<sup>z</sup>Shoot dry weight is the combination of stem and leaf dry weights.

<sup>y</sup>Means followed by the same letter within response and main effect are not different (Tukey's HSD;  $\alpha = 0.05$ ) and represent the average of two trials, five replicates and two irrigation treatments for rootstock main effect (n = 20 data points for each mean) and two trials, five replicates, and three rootstock treatments for the irrigation main effect (n = 30 data points for each mean).

note that this rootstock showed a general negative effect on plant growth and reduced  $g_S$  compared with the other treatments. Similarly, we found that S tended to reduce leaf area compared with C (Fig. 4). In contradiction with Nilsen et al. (2014), we do not consider this rootstock-induced reduction of leaf area a form of water conservation as relative water content and  $g_S$  remained the lowest in S regardless of reduced leaf area (Figs. 5 and 6B).

The  $\Phi_{PSII}$  was the only response for which the interaction of rootstock and irrigation was significant (Table 1). A general decrease in  $\Phi_{PSII}$  values was observed with reduced irrigation among the rootstocks but the magnitude of this decrease was much greater for S and C than B (Fig. 6C). Photosynthesis is one of the primary processes affected by low water status due either to limited assimilable CO<sub>2</sub>, the increase of reactive oxidative species, and/or metabolic changes (Chaves, 1991; Lawlor and Cornic, 2002). Photosystem II, I, and the electron transport chain can maintain functionality at reduced relative water content but the efficiency with which photosystem II uses absorbed photons ( $\Phi_{PSII}$ ) decreases with reduced relative water content. The higher  $\Phi_{PSII}$  observed in 'B' at reduced irrigation may be due to the overall higher relative water content associated with this rootstock (Figs. 5 and 6C).

Root:shoot ratio is a commonly used metric when measuring whole-plant responses to limited water. An increase in root:shoot ratio, as a result of preferential

carbon allocation to root system development, is routinely observed when soil resources are limited (Comas et al., 2013; Shipley and Meziane, 2002). We observed this root:shoot ratio increase in response to reduced irrigation due to a concomitant increase in root dry weight and reduction in shoot dry weight (Table 4). In agreement with Comas et al. (2013), there is limited scientific support to make inferences on root system morphology or water use efficiency based on root:shoot ratio. Because this metric is solely based on dry weight, it gives no information regarding root length, diameter, surface area, or any other physiologically relevant root system morphological trait. Furthermore, making root system inferences based on root:shoot ratio alone may be deceptive. For example, no difference in root:shoot ratio between S and B were observed; both had similar shoot and root dry weights (Table 4). However, B had a total root length  $\approx 25\%$  longer than S (116.8 m vs. 94.9 m, respectively; Fig. 7A), and a higher proportion of the root length of B was composed of very thin diameter roots (Table 3). None of this information can be inferred when comparing root:shoot ratios. As such, research focused on comparing traits or changes in root systems due to resource limitations should use a more information dense metric such as specific root length, which gives a measure of root length benefit to carbon investment in root system development.

## Conclusion

This study represents one of the first to compare tomato rootstock root morphology and scion morphology and physiology related to water availability. Water, or the lack thereof, is a growing challenge faced by all involved in agriculture and food production. It is a critical and grand challenge to develop crops that respond to limited available water in a manner that improves resilience and water use efficiency. The constitutive positive influence on shoot morphology and physiology and unique root system of 'Beaufort' rootstock, coupled with its documented improvement in production (Djidonou et al., 2013; Suchoff et al., 2018), make it a strong option for tomato production under reduced irrigation. Future work is warranted in comparing water use efficiency in rootstocks with similar root system morphologies to 'Beaufort', as well as investigating whether unique molecular or biochemical changes are associated with different rootstocks when grown in conditions of limited water.

## Literature Cited

- Altunlu, H. and A. Gul. 2012. Increasing drought tolerance of tomato plants by grafting. *Acta Hort.* 960:183–190.
- Al-Harbi, A., A. Hejazi, and A. Al-Omran. 2017. Responses of grafted tomato (*Solanum lycopersicon* L.) to abiotic stresses in Saudi Arabia. *Saudi J. Biol. Sci.* 24:1274–1280.
- Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? *Austral. J. Agr. Res.* 56:1159–1168.
- Bouma, T.J., K.L. Nielsen, and B.A.S. Koutstaal. 2000. Sample preparation and scanning protocol for computerized analysis of root length and diameter. *Plant Soil* 218:185–196.
- Bray, E. 1997. Plant responses to water deficit. *Trends Plant Sci.* 2:48–54.
- Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. *J. Expt. Bot.* 42:1–16.
- Comas, L.H., S.R. Becker, V.C. Von Mark, P.F. Byrne, and D.A. Dierig. 2013. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* 4:442.
- Deikman, J., M. Petracek, and J.E. Heard. 2012. Drought tolerance through biotechnology: Improving translation from the laboratory to farmers' fields. *Curr. Opin. Biotechnol.* 23:243–250.
- Djidonou, D., X. Zhao, E.H. Simonne, K.E. Koch, and J.E. Erickson. 2013. Yield, water-, and nitrogen-use efficiency in field-grown, grafted tomatoes. *HortScience* 48:485–492.
- Henry, A., A.J. Cal, T.C. Batoto, R.O. Torres, and R. Serraj. 2012. Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. *J. Expt. Bot.* 63:4751–4763.
- Hernández, E.I., A. Vilagrosa, J.G. Pausas, and J. Bellot. 2010. Morphological traits and water use strategies in seedlings of Mediterranean coexisting species. *Plant Ecol.* 207:233–244.
- Ho, M.D., J.C. Rosas, K.M. Brown, and J.P. Lynch. 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Funct. Plant Biol.* 32:737–748.
- Hsaio, T.C. 1973. Plant responses to water stress. *Annu. Rev. Plant Physiol.* 24:519–570.
- Huang, B. and D.M. Eissenstat. 2000. Linking hydraulic conductivity to anatomy in plants

- that vary in specific root length. *J. Amer. Soc. Hort. Sci.* 125:260–264.
- Ibrahim, A., M. Wahb-Allah, H. Abdel-Razzak, and A. Alsadon. 2014. Growth, yield, quality and water use efficiency of grafted tomato plants grown in greenhouse under different irrigation levels. *Life Sci. J.* 11:118–126.
- Kumar, P., Y. Roupael, M. Cardarelli, and G. Colla. 2017. Vegetable grafting as a tool to improve drought resistance and water use efficiency. *Front. Plant Sci.* 30:1130.
- Kyriacou, M.C., Y. Roupael, G. Colla, R. Zrenner, and D. Schwarz. 2017. Vegetable grafting: The implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. *Front. Plant Sci.* 8:741.
- Lawlor, D.W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* 25:275–294.
- Levitt, J. 1972. Responses of plants to environmental stresses. Vol. 2: Water, radiation, salt and other stresses. Academic Press, New York.
- Louws, F.J., C.L. Rivard, and C. Kubota. 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Scientia Hort.* 127:127–146.
- Malamy, J.E. 2005. Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ.* 28:67–77.
- Manavalan, L.P., S.K. Guttikonda, V.T. Nguyen, J.G. Shannon, and H.T. Nguyen. 2010. Evaluation of diverse soybean germplasm for root growth and architecture. *Plant Soil* 330:503–514.
- Mickelbart, M.V., P.M. Hasegawa, and J. Bailey-Serres. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nat. Rev. Genet.* 16:237–251.
- Molden, D. (ed.). 2007. Water for food, water for life: A comprehensive assessment of water management in agriculture. International Water Management Institute. Earthscan, London, UK.
- Nilsen, E.T., J. Freeman, R. Grene, and J. Tokuhisa. 2014. A rootstock provides water conservation for a grafted commercial tomato (*Solanum lycopersicum* L.) line in response to mild-drought conditions: A focus on vegetative growth and photosynthetic parameters. *PLoS One* 9:e115380.
- President's Council of Advisors on Science and Technology (PCAST). 2012. Report to the president on agricultural preparedness and the agriculture research enterprise. Executive Office of the President, President's Council of Advisors on Science and Technology, Washington DC.
- Rivard, C.L. and F.J. Louws. 2006. Grafting for disease resistance in heirloom tomatoes. North Carolina Coop. Ext. Serv. Bul. Ag-675. NC State Univ., Raleigh, NC.
- Roupael, Y., M.C. Kyriacou, and G. Colla. 2018. Vegetable grafting: A toolbox for securing yield stability under multiple stress conditions. *Front. Plant Sci.* 8:2255.
- Schwarz, D., Y. Roupael, G. Colla, and J.H. Venema. 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Scientia Hort.* 127:162–171.
- Shao, H.-B., L.-Y. Chu, C.A. Jaleel, and C.-X. Zhao. 2008. Water-deficit stress-induced anatomical changes in higher plants. *C. R. Biol.* 331:215–225.
- Shipley, B. and D. Meziane. 2002. The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. *Funct. Ecol.* 16:326–331.
- Somerville, C. and J. Briscoe. 2001. Genetic engineering and water. *Science* 292:2217.
- Suchoff, D.H., C.C. Gunter, and F.J. Louws. 2017. Comparative analysis of root system morphology in tomato rootstocks. *HortTechnology* 27:319–324.
- Suchoff, D.H., J.R. Schultheis, M.D. Kleinhenz, F.J. Louws, and C.C. Gunter. 2018. Rootstock improves high-tunnel tomato water-use efficiency. *HortTechnology* 28:344–353.
- Trenberth, K.E. 2011. Changes in precipitation with climate change. *Clim. Res.* 47:123–138.
- The United Nations. 2000. Secretary-general, in address to developing countries “south summit”, calls for steps to make global economy more equitable [Press release]. 21 Mar. 2018. <<https://www.un.org/press/en/2000/20000412.sgsn7358.doc.html>>.
- United States Department of Agriculture Economic Research Service. 2010. U.S. Tomato Statistics; State census farms with tomatoes, area harvested, & area irrigated, 1978-2007. 21 Mar. 2018. <<http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1210>>.
- Wasson, A.P., R.A. Richards, R. Chatrath, S.C. Misra, S.V. Prasad, and G.J. Rebetzke. 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *J. Expt. Bot.* 63:3485–3498.
- White, J.W. and J.W. Mastalerz. 1966. Soil moisture as related to “container capacity.” *Proc. Amer. Soc. Hort. Sci.* 89:758–776.