ABSTRACT

SUCHOFF, DAVID H. Rooting for Resilience: Evaluation of Abiotic Stress Tolerance in Tomato Rootstocks. (Under the direction of Dr. Christopher C. Gunter).

Grafting tomatoes (*Solanum lycopersicum*) onto resistant rootstocks is an increasingly popular practice for managing numerous economically damaging soil-borne pathogens. The main driver for the use of grafted tomatoes is this rootstock-derived disease resistance. However, a growing body of research has shown that rootstocks have the ability to improve abiotic stress tolerance and soil resource use efficiency. A research gap exists in current scientific understanding of the mechanisms that confer these additional rootstock benefits. As such, the overall objectives of this body of research was to characterize the root system morphology of numerous commercially available tomato rootstocks and determine which of these traits allow for improved water-use efficiency and suboptimal temperature tolerance. Significant differences in total root length, average root diameter, specific root length, and diameter class proportions were elucidated among 17 commercially available tomato rootstocks and one commercial field cultivar. Two rootstocks with differing root system morphologies (‘Beaufort’ and ‘Shield’) were chosen for an on-farm study to determine whether they could improve the water-use efficiency of ‘Cherokee Purple’ when used as a scion compared to a non-grafted ‘Cherokee Purple’ controls. Grafted treatments were given either 100% (3 h every other day) or 50% (1.5 h every other day) of the grower’s traditional irrigation regime. Plants grafted onto ‘Beaufort’ showed no yield reduction at the 50% irrigation treatment and out yielded the non-grafted control at 100% irrigation. An economic determined the feasibility of utilizing grafted plants in conditions lacking significant disease pressure. The increased yield obtained when utilizing ‘Beaufort’ rootstock at 50% irrigation increased net revenue by $35,900 per acre compared to non-grafted ‘Cherokee Purple’
receiving 100% irrigation, amounting to a 44.6% increase in net revenue while saving approximately 383,242 gal/acre of water per growing season.

Further exploration of this performance enhancement required a greenhouse dry-down study to determine how these different rootstocks respond to drying soils. A constitutive positive increase on relative water content, leaf area, stomatal conductance, and net CO₂ assimilation rate was observed with ‘Beaufort’. This rootstock had a significantly longer total root system (118.6 m) compared to ‘Shield’ (94.9 m) and the self-grafted ‘Cherokee Purple’ control (104.2 m). Furthermore, 76.4% of the total root length observed in ‘Beaufort’ was composed of very thin diameter roots (Ø < 0.5 mm), which was higher (p<0.05) than ‘Shield’ (73.7%) and the self-grafted control (69.1%). The ability for ‘Beaufort’ to maintain longer total root length composed of very thin diameter roots may increase absorptive capacity and root hydraulic conductance, thus allowing for the observed higher relative water content, leaf area, and stomatal conductance. A final study was conducted to determine whether commercially available tomato rootstocks with differing parental backgrounds and root system morphologies could improve the tolerance of scion plants to suboptimal temperature. Two controlled environment growth chambers were utilized and maintained at either optimal (25 °C day / 20 °C night) or suboptimal (15 °C day / 15 °C night) temperatures. The cold-sensitive tomato cultivar Moneymaker was used as the non-grafted and self-grafted control as well as scion on ‘Multifort’, ‘Shield’, and S. habrochaites LA 1777 rootstocks. ‘Multifort’ rootstock reduced the amount of cold-induced leaf area reduction and maintained higher levels of CO₂ assimilation and photosystem II quantum efficiency. ‘Multifort’ had longer roots, having 42% to 56% more fine root (diameter less than 0.5 mm) length compared to the other rootstock treatments. Leaf starch
concentration was lower in ‘Multifort’-grafted plants at suboptimal temperatures. The ability for ‘Multifort’ to maintain root growth at suboptimal temperatures may improve root system sink strength, thus allowing for proper movement of photosynthate from leaf to root even under cold conditions.

The results from this work indicate that a new layer of customizability may be available to growers utilizing grafted plants; scions can be chosen to meet market demand while rootstocks could be selected to meet the biotic and abiotic pressures of their production environment. These additional rootstock-derived benefits may allow for more environmentally friendly and economically advantageous options in the production of field- and greenhouse-grown tomatoes.
Rooting for Resilience: Evaluation of Abiotic Stress Tolerance in Tomato Rootstocks

by

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Horticultural Science

Raleigh, North Carolina

2018

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DEDICATION

To my family- Debbie, Michael, Sam, Catlin, and Sebastian.
BIOGRAPHY

David H Suchoff was born on December 4, 1984 to Michael and Debbie Suchoff. David spent the first years of his life in Los Angeles, CA where his father worked as an electrical engineer and mother as an artist. In 1994 David and his family moved to Carrboro, NC. In 2003 David went to the University of North Carolina at Chapel Hill for his undergraduate education majoring in Music Performance with a chemistry minor. Upon his graduation in December 2007, David applied to the Peace Corps. In August 2008 David left for Paraguay to work as an agricultural extension agent for the Peace Corps. His work in Paraguay included promoting farming methods that would make the current practices more sustainable and productive. Additionally, he worked with many women’s committees to help develop family and committee gardens that supplied families with nutritious fruits and vegetables year-round. Working at the local school, David developed a school garden and tree nursery for educational purposes as well as a means to provide students with nutritious snacks and to reforest the community. Having completed his two-year service, David was offered the opportunity to continue working with the Peace Corps for a third year in Costa Rica. Here David continued with similar work; however, he focused more on home garden development as well as training of other Peace Corps volunteers in sustainable agriculture development and teaching techniques.

David returned to the US in January 2012 to work as an apprentice on the Small Farm Unit at the Center for Environmental Farming Systems (CEFS) in Goldsboro, NC. Here David lived and worked for a year learning how to produce fruits, vegetables, meat, eggs, and dairy on a 50-acre small farm. Having worked with faculty from North Carolina State University (NCSU) conducting research at CEFS, it became apparent to David that he
wanted to focus on horticulture and study at NCSU. He received a Master’s of Science in May 2015. His research focused on vegetable production; specifically, the nitrogen use efficiency of grafted tomatoes and grafted watermelon. David continued in this area of research for his Ph.D., focusing on resource use efficiency and abiotic stress tolerance in grafted tomatoes under the guidance of Dr. Christopher Gunter.
ACKNOWLEDGMENTS

This work would not have been possible without the guidance of my committee chair Dr. Chris Gunter and committee members Drs. Frank Louws, Matthew Kleinhenz, Heike Sederoff, and Jonathan Schultheis.

I would like to thank Betsy and Alex Hitt as well as Jenny Rasmussen of Peregrine Farm who allowed us to conduct our on-farm study.

The studies that comprise this dissertation represent the sum of countless hours of data collection for which I had significant technical assistance. Matthew Waldschmidt, Rebecca Eskalis, Courtney DeKalb, Abigail Dexter-Boone, and Matthew Bertucci played critical roles in data collection, especially the arduous process of cleaning and collecting roots.

Finally, I must thank my family who have been strong supporters of my academic endeavors.
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CHAPTER 1

Comparative Analysis of Root System Morphology in Tomato Rootstocks

(As published in HortTechnology 27(3), pp. 319-324)
Comparative Analysis of Root System Morphology in Tomato Rootstocks

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S Department of Agriculture, under award number 2011-01397.

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Comparative Analysis of Root System Morphology in Tomato Rootstocks

*Additional index words.* *Lycopersicum esculentum;* WinRHIZO; grafted; specific root length; average root diameter; total root length

*Summary.* At its most basic, grafting is the replacement of one root system with another containing more desirable traits. Grafting of tomato (*Solanum lycopersicum*) onto disease-resistant rootstocks is an increasingly popular alternative for managing economically damaging soil-borne diseases. Although certain rootstocks have demonstrated ancillary benefits in the form of improved tolerance to edaphic abiotic stress, the mechanisms behind the enhanced stress tolerance are not well understood. Specific traits within root system morphology (RSM), in both field crops and vegetables, can improve growth in conditions under abiotic stress. A greenhouse study was conducted to compare the RSM of 17 commercially available tomato rootstocks and one commercial field cultivar (Florida-47). Plants were grown in containers filled with a mixture of clay-based soil conditioner and pool filter sand (2:1 v/v) and harvested at 2-, 3-, or 4-weeks after emergence. At harvest, roots were cleaned, scanned, and analyzed with an image analysis system. Data collected included total root length (TRL), average root diameter, specific root length, and relative diameter class. The main effect of cultivar was significant (*P* ≤ 0.05) for all response variables and the main effect of harvest date was only significant (*P* ≤ 0.01) for TRL. ‘RST-106’ rootstock had the longest TRL whereas ‘Beaufort’ had the shortest. ‘BHN-1088’ had the thickest average root diameter, which was 32% thicker than the thinnest, observed in ‘Beaufort’. SRL in ‘Beaufort’ was 60% larger than ‘BHN-1088’. This study demonstrated that gross differences exist in RSM of tomato rootstocks and that, when grown in a solid porous medium, these differences can be determined using an image analysis system.
The efficacy of herbaceous grafts relies on the replacement of a scion root system with that of a rootstock with known disease resistance. The use of disease resistant rootstocks in grafted tomato production has proven efficacious in managing numerous economically significant soil-borne diseases (Kubota et al., 2008; Kunwar et al., 2015; Lee and Oda, 2002; Louws et al., 2010). In addition, certain rootstocks can improve tolerance to abiotic stress such as cold soils, salinity, drought, and flooding (Albacete et al., 2015; Colla et al., 2006; Djidonou et al., 2013; Estañ et al., 2005; He et al., 2009; Venema et al., 2008; Yetisir et al., 2006). These additional benefits afforded by certain rootstocks can allow a grower to custom tailor the scion-rootstock combination to their local production environment and disease pressure.

Tolerance to edaphic stress has been linked to root system morphology (RSM). Increased total root length (TRL) has been attributed to improving nutrient uptake, especially phosphorus (P), when availability is low (Hill et al., 2006; Lambers et al., 2006). In drought conditions, increased TRL, particularly in the deeper soil profile, can improve water acquisition (Comas et al., 2013; Ho et al, 2005; Lopes and Reynolds, 2010; Schenk and Jackson, 2005; Wasson et al., 2012). A smaller root diameter may also aid water uptake in dryer conditions by reducing hydraulic resistance (Huang and Eissenstat, 2000; Passioura, 1988; Rieger and Litvin, 1999; Sharp et al., 1988; Steudle and Peterson, 1997). A reduction in root diameter has also been observed in response to low P concentrations (Hill et al., 2006; Zobel et al., 2007) and salinity (Lovelli et al., 2012).

Specific root length (SRL), defined as a proportion of TRL to root dry matter, is a metric used to describe the ratio of the morphological benefit to metabolic cost in root system development (Eissenstat, 1992). An increase in SRL, in relation to a reduction of root
diameter, has been observed as a response to low P (Christy and Moorby, 1975; Hill et al., 2006; Lambers et al., 2006; Schroeder and Janos, 2005), drought (Huang and Eissenstat, 2000) and salinity (Lovelli et al., 2012).

Though both intrinsic RSM and the changes observed in response to abiotic stress and reduced resources have been well studied, limited research has been conducted to compare tomato root systems. Differences by cultivar have been observed in the RSM of processing tomatoes (Portas and Dordio, 1980; Zobel, 1975). For tomato rootstock root systems, one hydroponic study comparing two commercial rootstocks (‘Beaufort’ and ‘Heman’) indicated differences in root density but not of average root diameter (Oztekin et al., 2009). The physical aspects of solid substrates can greatly affect root systems grown in soil compared to those in hydroponics (Chapman et al., 2012). The spatial heterogeneity of nutrient and water content within solid substrates affects both root morphology and architecture (Desnos, 2008; Forde and Lorenzo, 2001; Lopez-Bucio et al., 2003). Hydroponic systems are designed to optimize growth by supplying roots with homogenous root zone resources. Consequently, translation of results from hydroponic studies, especially solution-based systems, to plants grown in the field or solid substrate should be done so with caution. To date, no research has been conducted comparing commercial tomato rootstock RSM in substrate-grown plants.

The goal of this study was to assess tomato rootstock RSM and development in solid substrates. This information may help characterize rootstocks for their potential increase in abiotic stress tolerance observed and aid in screening and/or breeding for stress tolerance in tomato rootstocks. The specific objectives of this study were: (1) compare RSM of 17 commercially available rootstocks and one commonly used tomato cultivar grown in a
pores, solid substrate, at the seedling stage and (2) determine how RSM in these cultivars changes over time.

Materials and Methods

This experiment was conducted in the Marye Anne Fox Science Teaching Laboratory Greenhouses on North Carolina State University Campus (NCSU), Raleigh, NC between 15 Oct. 2015 and 21 Nov. 2015. The second trial of the study was conducted between 23 Nov. 2015 and 3 Jan. 2016. Seventeen commercially available tomato rootstocks and one common determinate tomato cultivar (Florida 47; Seminis Vegetable Seeds, Inc., St. Louis, MO) were used (Table 1).

Three seeds of each cultivar were planted 2 mm deep in 2.8 L black polyethylene pots with dimensions of 6 inches (top diameter) x 7 inches (height) x 5 inches (bottom diameter) (Poly-Tainer #1; Hummert International, Earth City, MO). Upon emergence, seedlings were thinned to one plant per pot. Pots were lined with woven 20 × 20 mesh of 0.02 cm diameter thread (~0.016 cm² opening size) (Clear Advantage Charcoal Fiber Glass Insect Screen; New York Wire, Hanover, PA) and filled with a 2:1 v/v mixture of clay-based soil conditioner (Turface MVP; Profile Products LLC, Buffalo Grove, IL) and sand (#20 Pool Filter Sand; Aquabrite®, Pleasanton, CA). This mixture was chosen as it provides a rooting medium more similar to that of the field while still allowing for easy separation and cleaning of the medium from roots (Manavalan et al., 2010). The mesh liner was used to prevent the medium from falling through the large drainage holes in the container as well as aid in root harvest.

Plants were destructively harvested based on chronological age at 2, 3, or 4 weeks after emergence, which corresponded to the appearance of the first set of true leaves, the full
expansion of the first two true leaves and the appearance of the second set of true leaves, and the full expansion of the second set of true leaves, respectively. This resulted in 54 unique treatments (18 cultivars × 3 harvest dates). The experiment followed a randomized complete block design with four blocks each containing all 54 unique treatments. Each block was arranged north to south on a greenhouse bench to take into account potential variation due to sunlight gradients. Since rootstocks differed in date to emergence, the date of emergence from the soil was noted and harvest date was calculated accordingly. Greenhouse temperatures during the day were maintained at 26.7 ± 4 °C and 18.3 ± 3 °C at night. Watering occurred every 3 d with fertilizing applied via irrigation (200 mg·L⁻¹ concentration of 20 nitrogen – 4.4 phosphorus – 16.6 potassium; Peters Professional, JR Peters Inc., Allentown, PA) once per week.

At the time of harvest plants and medium were pulled from the container with the aid of the mesh liner. Plants were gently excavated by hand from the medium. Once the root system was freed, the medium was thoroughly examined for any roots that may have broken off during the processing. All roots were water-rinsed of any remaining medium and placed in a container filled with 10 mL of 0.5 g·L⁻¹ neutral red stain (Sigma Aldrich Co., St. Louis, MO) and stored for 24 h at 6.7 °C. The staining process was imposed to improve contrast and overall resolution during the scanning process as recommended by Bouma et al. (2000).

Following the staining process, roots were thoroughly rinsed for 3 min in deionized (DI) water prior to scanning. A 30 x 42 cm acrylic tray was placed on top of a flatbed scanner (Epson Expression® 10000XL; Epson America, Long Beach, CA) and filled with ~ 2 cm of DI water. Roots were placed in the tray and gently positioned with no overlapping roots to allow for more uniform scanning. Scans were done in grey scale at 800 dots per inch to
increase resolution of fine roots. Each image was analyzed using WinRHIZO™ version 2012b image analysis system (Regent Instruments Inc., Quebec, Canada). Image analysis data collected included TRL, average root diameter, and length per diameter class (diameter classes were in increments of 0.5 mm). Length per diameter class data were normalized by dividing by TRL, resulting in a ratio of diameter class root length per TRL (relative diameter class). Following scanning, roots were dried at 70 °C for 24 h (Thelco 130D Laboratory Oven; Precision Scientific Co., Winchester, VA) and dry weights of the total root system were taken (AE100 Digital Analytical Scale; Mettler-Toledo LLC, Columbus, OH). Dry weight measurements were used to calculate specific root length (TRL/total dry weight).

Data from both trials were combined and analyzed with PROC MIXED in SAS (version 9.4; SAS Institute Inc. Cary, NC). The model used to analyze all data contained cultivar, harvest date, and their interaction as fixed effects with trial and block nested in trial as random effects. Residual plots were studied for any violation of the assumptions in analysis of variance (ANOVA) such as heterogeneity and outliers. No outliers were observed, however, residual plots for TRL and relative diameter class showed strong heteroscedasticity. An arcsine and log conversion were imposed on relative diameter class and TRL data, respectively, in order to homogenize residual variance. For reporting, data were back-transformed. When appropriate, Tukey’s honest significant difference (HSD) was used as a post hoc mean separation test.

Results

Initial analysis was conducted by experiment, however, there was no significant experiment × treatment interaction. Consequently, data from both experiments were
combined. No significant cultivar × harvest date interactions were found for any of the response variables (Table 2). The main effect of harvest date was significant only for TRL ($P \leq 0.01$) which increased with harvest date (Fig. 1). TRL was also significantly affected ($P \leq 0.05$) by rootstock. ‘RST-106’ had the longest TRL with ‘Beaufort’ having the shortest (Fig. 2). These two rootstocks represent the extremes in TRL with the remaining 16 cultivars falling in between as intermediate in their TRL. For all other response variables the main effect of cultivar was significant at $P \leq 0.001$ (Table 2). Average root diameter was narrowest in ‘Beaufort’ (0.28 mm), ‘TD-1’ (0.29 mm), ‘Kaiser’ (0.29 mm), ‘RST-105’ (0.29 mm), ‘Multifort’ (0.30 mm), and ‘Emperador’ (0.30 mm) (Fig. 3). ‘BHN-1088’ had the widest average root diameter (0.37 mm) compared to all other cultivars. All remaining 11 cultivars were intermediate in the average root diameter compared to those found in the extremes.

Specific root length was largest in ‘Beaufort’ (40,124 cm·g$^{-1}$), ‘TD-1’ (40,056 cm·g$^{-1}$), ‘Multifort’ (39,333 cm·g$^{-1}$), and ‘Kaiser’ (37,967 cm·g$^{-1}$) (Fig 4). ‘BHN-1088’ (25,147 cm·g$^{-1}$), ‘Camel’ (26,147 cm·g$^{-1}$), and ‘Cheong Gang’ (26,996 cm·g$^{-1}$) were among the cultivars that had the lowest SRL.

The majority of the total root length for all cultivars fell into diameter class 1 (≤ 0.5 mm) (Table 3). Within this relative diameter class, ‘Beaufort’ had the highest proportion (0.9625) followed by ‘RST-105’ (0.9524), ‘Multifort’ (0.9488), and ‘Kaiser’ (0.9458). ‘BHN-1088’ had the lowest proportion (0.8661) of TRL in this relative diameter class. Results for relative diameter class 2 (0.5 – 1.0 mm) were opposite that of relative diameter class 1 – ‘BHN-1088’ had the highest proportion (0.1266) of TRL with ‘Beaufort’ (0.0361), ‘RST-105’ (0.0459), ‘Multifort’ (0.0498) and ‘Kaiser’ (0.0514) having the lowest proportion in relative diameter class 2.
Discussion

This research indicates that quantifiable morphological differences exist between tomato rootstock root systems. Some of the differences observed may explain the improved stress tolerance provided by specific tomato rootstocks. When used as rootstocks for grafted ‘Florida 47’, both ‘Multifort’ and ‘Beaufort’ improved water use efficiency compared to non-grafted ‘Florida 47’ (Djidonou et al, 2013). The authors suggested that this improved water use efficiency may be due to root morphology. Our results with these cultivars show that SRL in Beaufort and Multifort were 41% and 38% greater than ‘Florida 47, respectively (Fig. 4). This difference is due to both ‘Beaufort’ and ‘Multifort’ having significantly thinner average root diameters than ‘Florida 47’. High SRL has been shown to increase hydraulic conductance in a trifoliate orange (Poncirus trifoliata) rootstock (Huang and Eissenstat, 2000). The authors attributed this increased hydraulic conductivity to the increased radial conductivity of the thinner rooted trifoliate orange. The increase in water use efficiency observed by Djidonou et al. (2013) may be due to the increased radial conductivity of thinner rooted ‘Beaufort’ and ‘Multifort’ rootstocks, allowing for increased hydraulic conductivity even with reduced irrigation.

At low levels of salinity (22 mM sodium chloride) grafting of the tomato cultivar Belladona onto ‘Beaufort’ improved yields compared to non-grafted controls (Savvas et al., 2011). Reduction of average root diameter and consequent increase in specific root length has been demonstrated to be a response in tomatoes to increasing levels of salinity (Lovelli et al., 2012). The authors hypothesized that the increased specific root length allows for osmotic adjustment without a large investment in carbon partitioned to the roots. Moreover, they concluded that the increase in specific root length may be an adaption to increase overall root
surface area, aiding in water and nutrient uptake in saline conditions. The improved yield in tomatoes with ‘Beaufort’ rootstock at low levels of salinity also coincided with an increase in leaf calcium concentrations (Savvas et al., 2011). A separate study found that ‘Beaufort’ rootstock improved uptake of nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur compared to self-grafted controls (Leonardi and Giuffrida, 2006). The high specific root length observed in ‘Beaufort’ in our study may aid in improving nutrient uptake due to increased surface area.

Recent evidence suggests that shoot-derived compounds can alter root morphology (Spiegelman et al., 2015). Our study analyzed root systems from non-grafted plants. Future work is warranted to determine if scion selection alters rootstock RSM. Furthermore, studies are needed to elucidate whether the morphological traits observed in this study are static or plastic with changing edaphic environments and what the relative role RSM plays compared to physiological mechanisms in stress.

Tomato rootstocks offer growers the ability to manage soil-borne diseases and ameliorate the negative effects of edaphic stress. This study demonstrates that root system morphology in tomato rootstocks differs by cultivar and remains similar over time, other than total root length. These differences may help explain the improved growth and production associated with specific rootstocks and could be used to classify cultivars for their suitability for use in specific growing conditions. Furthermore, the use of a porous medium coupled with scanning and analysis using an image analysis system allows for a detailed analysis of roots for plants grown in a solid substrate.
Literature cited:


Table 1. List of seed companies, their locations, and tomato (*Solanum lycopersicum*) cultivars used in this experiment.

<table>
<thead>
<tr>
<th>Company</th>
<th>City, State</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakata</td>
<td>Morgan Hill, CA</td>
<td>FTM2492</td>
</tr>
<tr>
<td>Rijk Zwaan</td>
<td>Salinas, CA</td>
<td>Emperador</td>
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<td></td>
<td></td>
<td>Kaiser</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shield RZ</td>
</tr>
<tr>
<td>DP Seeds</td>
<td>Yuma, AZ</td>
<td>RST-04-106-T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RST-04-105-T</td>
</tr>
<tr>
<td>De Ruiter</td>
<td>St. Louis, MO</td>
<td>Shincheong gang</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cheong gang</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beaufort</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multifort</td>
</tr>
<tr>
<td>BHN Seed</td>
<td>Immokalee, FL</td>
<td>BHN 1087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BHN 1088</td>
</tr>
<tr>
<td>American Takii, Inc</td>
<td>Salinas, CA</td>
<td>Armada</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B.B. Camel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TD-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TD-2</td>
</tr>
<tr>
<td>Seminis Vegetable Seeds</td>
<td>St. Louis, MO</td>
<td>Florida 47</td>
</tr>
</tbody>
</table>
Table 2. Results from a two-way ANOVA for a fully factorial arrangement (18 cultivar x 3 harvest dates) of treatments for the combined experimental replicates.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Total root length</th>
<th>Average diameter&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Dclass1&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Dclass2&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Specific root length&lt;sup&gt;w&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>17</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Harvest date</td>
<td>2</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>34</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at $P \leq 0.05$, significant at $P \leq 0.05$, 0.01, 0.001, respectively.

<sup>z</sup>Average root diameter for an entire root system.

<sup>y</sup>Relative diameter class calculated as total length of roots of diameter < 0.5 mm as a proportion of total root length.

<sup>x</sup>Relative diameter class calculated as total length of roots of diameter between 0.5 and 1.0 mm as a proportion of total root length. 1 mm = 0.0394 inch.

<sup>w</sup>Total root length divided by root dry weight.
Table 3. Main effect of tomato (*Solanum lycopersicum*) rootstock on proportional distribution of root diameter class.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Diameter class 1 (&lt;0.5 mm)</th>
<th>Diameter class 2 (0.5-1.0 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaufort</td>
<td>0.9625 a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.0361 e</td>
</tr>
<tr>
<td>RST-105</td>
<td>0.9524 ab</td>
<td>0.0459 de</td>
</tr>
<tr>
<td>Multifort</td>
<td>0.9488 abc</td>
<td>0.0498 cde</td>
</tr>
<tr>
<td>Kaiser</td>
<td>0.9458 abcd</td>
<td>0.0514 cde</td>
</tr>
<tr>
<td>Emperador</td>
<td>0.9417 abcd</td>
<td>0.0564 bcde</td>
</tr>
<tr>
<td>TD-1</td>
<td>0.9416 abcd</td>
<td>0.0568 bcde</td>
</tr>
<tr>
<td>B.B.</td>
<td>0.9148 bcde</td>
<td>0.0814 abcd</td>
</tr>
<tr>
<td>Shincheong Gang</td>
<td>0.9142 bcde</td>
<td>0.0817 abcd</td>
</tr>
<tr>
<td>Armada</td>
<td>0.9124 bcde</td>
<td>0.0844 abcd</td>
</tr>
<tr>
<td>BHN1087</td>
<td>0.9108 bcde</td>
<td>0.0853 abcd</td>
</tr>
<tr>
<td>TD-2</td>
<td>0.9043 cde</td>
<td>0.0901 abcd</td>
</tr>
<tr>
<td>FTM2492</td>
<td>0.9026 de</td>
<td>0.0924 abc</td>
</tr>
<tr>
<td>RST-106</td>
<td>0.8957 e</td>
<td>0.0994 ab</td>
</tr>
<tr>
<td>Florida 47</td>
<td>0.8925 e</td>
<td>0.1032 a</td>
</tr>
<tr>
<td>Shield RZ</td>
<td>0.8869 e</td>
<td>0.1076 a</td>
</tr>
<tr>
<td>Camel</td>
<td>0.8794 e</td>
<td>0.1133 a</td>
</tr>
<tr>
<td>Cheong Gang</td>
<td>0.8719 e</td>
<td>0.1206 a</td>
</tr>
<tr>
<td>BHN1088</td>
<td>0.8661 e</td>
<td>0.1266 a</td>
</tr>
</tbody>
</table>

<sup>2</sup>Means followed by the same letter within a diameter class are not significantly different (Tukey’s HSD; α = 0.05) and represent the average of four replicate samples, three harvest dates, and two repeated experiments (n=24 data points for each mean); 1 mm = 0.0394 inch.
Figure. 1. Main effect of harvest in tomato (*Solanum lycopersicum*) rootstock total root length ± standard error over time. Means with common letters are not different (Tukey’s HSD; $\alpha = 0.05$) and represent the mean of four replicate samples, 18 cultivars, and two repeated experiments (n=144 data points for each mean); 1 cm = 0.3937 inch.
Figure 2. Main effect of tomato (Solanum lycopersicum) rootstock on total root length ± standard error. Means with common letters are not different (Tukey’s HSD; α = 0.05) and represent the average of four replicate samples, three harvest dates, and two repeated experiments (n=24 data points for each mean); 1 cm = 0.3937 inch.
Figure 3. Main effect of tomato (*Solanum lycopersicum*) rootstock on average root diameter ± standard error by cultivar. Means with common letters are not different (Tukey’s HSD; $\alpha = 0.05$) and represent the average of four replicate samples, three harvest dates, and two repeated experiments (n=24 data points for each mean); 1 mm = 0.0394 inch.
Figure 4. Main effect of tomato (*Solanum lycopersicum*) rootstock on specific root length ± standard error by cultivar. Means with common letters are not different (Tukey’s HSD; $\alpha = 0.05$) and represent the average of four replicate samples, three harvest dates, and two repeated experiments (n=24 data points for each mean); 1 cm·g$^{-1}$ = 11.1612 inches·oz$^{-1}$. 
CHAPTER 2

Rootstock Improves High-Tunnel Tomato Water-Use Efficiency
Rootstock Improves High-Tunnel Tomato Water-Use Efficiency

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S Department of Agriculture, under award number 2016-51181-25404.

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Subject Category: Vegetable Crops

Rootstock Improves High-Tunnel Tomato Water-Use Efficiency

*Additional index words.* Grafted tomatoes; herbaceous graft; irrigation; heirloom tomato; on-farm

Summary. The following study was conducted to address water use efficiency in grafted tomato (*Solanum lycopersicum*) in an on-farm environment. The commercial rootstock cultivars Beaufort and Shield were chosen as these two have different root system morphologies that may benefit water use efficiency. The heirloom cultivar Cherokee Purple was grafted onto both rootstocks as well as utilized as the non-grafted control. The study was conducted in 2016 and 2017 on a 5-acre vegetable and cut flower farm in North Carolina’s Piedmont region. Plants were grown under protected, high-tunnel culture where they received either 100% (3 h every other day) or 50% (1.5 h every other day) of the grower’s normal irrigation regime. At 50% irrigation, ‘Beaufort’-grafted plants yielded significantly more than non-grafted ‘Cherokee Purple’ and ‘Shield’-grafted plants receiving the same irrigation treatment as well as the non-grafted ‘Cherokee Purple’ receiving the 100% irrigation treatment. The ‘Beaufort’ grafted plants significantly improved irrigation water use efficiency (iWUE) at the 50% irrigation treatment compared to the other rootstock treatments. Yield and iWUE of ‘Shield’-grafted plants were comparable to the non-grafted ‘Cherokee Purple’ at both irrigation treatments. Regardless of irrigation treatment, grafting onto ‘Beaufort’ improved the quality of total fruit harvested. An economic assessment was conducted to determine the feasibility of utilizing grafted plants in conditions lacking significant disease pressure. Purchasing grafted transplants would increase the initial investment by $5,227.2 per acre. However, the increased yield obtained when utilizing ‘Beaufort’ rootstock at 50% irrigation increased net revenue by $35,900.41 per acre
compared to non-grafted ‘Cherokee Purple’ receiving 100% irrigation, amounting to an 44.6% increase in net revenue while saving approximately 383,242 gal/acre of water per growing season. These results indicate that growers can select rootstocks to better manage water use in an environmentally friendly manner without limiting economic gains.

Crop production systems must become more water use efficient. Approximately 70% of global and 80% of the United States fresh water consumption is for agricultural practices (Evans and Sadler, 2008; Schaible and Aillery, 2012). With continual growth in global population, it is estimated that, utilizing current practices, water consumption for agriculture will increase by 70% - 90% by 2050 (Molden, 2007). With the depletion of many fresh water sources and the increasing uncertainty of rainfall due to climate change, continuous increase in agricultural water consumption is untenable (FAO, 2002). These challenges have many calling for a “Blue Revolution” – the development of crops that can maintain or improve current yields while reducing overall water inputs (Pennisi, 2008).

Numerous cultural practices and breeding efforts have been aimed at improving water use efficiency. Practices that direct irrigation water to the plant roots, such as subsurface drip, or that mitigate surface evaporation can greatly improve water use efficiency by reducing water loss (De Pascale et al., 2011). Traditional breeding efforts for more water use efficient crops have shown mixed results. Many of the physiological plant responses associated with improved growth or maintenance at low water availability such as stomatal closure and reduced leaf area limit photosynthesis and consequently reduce yields (Blum, 2005).

Molecular approaches to improving water use efficiency show promise, but because of the complex network of genes associated with plant-water relations, few genetically altered crops show improved water use efficiency when grown in the field compared to
controlled environments (Pennisi, 2008). Root systems, being the site of water uptake, have garnered substantial attention in the effort to improve water uptake and use efficiencies (Gewin, 2010; Lynch, 2007). Numerous root morphological and architectural traits such as diameter, length, and spatial distribution all affect water uptake under limiting conditions (Comas et al., 2013; Ho et al., 2005; Huang and Eissenstat, 2000; Mickelbart et al., 2015). Developing crops that contain both desired fruit and root traits through traditional breeding efforts is difficult due to the necessary time, challenges with phenotyping root systems, and low heritability of root traits (Malamy, 2005; Wasson et al., 2012). Molecular approaches to altering root system traits may be a quicker means, compared to traditional breeding, but public perceptions of genetically modified crops may hinder widespread acceptance. One new approach not involving genetic modification is through grafting.

Grafting susceptible scion cultivars onto resistant rootstocks is an effective means of managing many economically significant soilborne pathogens that affect tomato (Louws et al., 2010). Though the main driver for grafting is the imparted resistance, numerous studies indicate that rootstocks may improve edaphic abiotic stress tolerance (Schwarz et al., 2010). Grafting the tomato cultivar Florida 47 onto either Multifort or Beaufort rootstocks improved irrigation water use efficiency (iWUE) in a two-year field study (Djidonou et al., 2013a). The authors hypothesized that the improved iWUE may be attributed to changes in root system morphology under stress. Root system morphoplasticity under stress can improve growth in limiting conditions; however, intrinsic root system morphological traits play a significant role in a plant's ability to efficiently utilize resources (Comas et al., 2013; Eissenstat, 1992; Henry et al., 2011). Both ‘Multifort’ and ‘Beaufort’ are interspecific hybrids (S. lycopersicum × S. habrochaites) and were shown to have similar root system morphologies that differed from
‘Florida 47’ (Suchoff et al., 2017). Whether the improved iWUE observed by Djidonou et al. (2013a) was due to differences in rootstock root system morphology (RSM) or grafting per-se is unclear. Furthermore, this study was conducted in an open field, controlled research facility setting. To date no study has compared iWUE of grafted tomatoes in an on-farm setting. To address this, the following study was conducted to: (1) Compare yield, growth, and iWUE of tomatoes grafted on rootstocks with differing RSM, and (2) determine if the use of grafted plants is economically beneficial in conditions lacking soilborne disease pressure. We hypothesize that those plants grafted onto ‘Beaufort’ rootstock will improve iWUE and yield at reduced irrigation due to its thin average root diameter and high specific root length (Suchoff et al., 2017), as these RSM traits have been shown to improve water use efficiency in other crops (Comas et al., 2013; Ho et al., 2005; Huang and Eissenstat, 2000).

Materials and Methods

Trial Location: This study was conducted during the summers of 2016 and 2017 on a commercial farm located in Alamance County, North Carolina. Soil samples were taken prior to transplanting the first trial in 2016. Results indicated Cecil sandy loam soil type with 0.56% humic matter, cation exchange capacity of 10.1 meq/100 cm³, and pH of 6.3. The growers use a multibay high-tunnel system (96 ft length × 24 ft width × 13 ft height Pioneer Series high tunnel; Haygrove Inc., Mount Joy, PA) for all tomato production to reduce foliar disease incidence and extend harvests. Consequently, all plant-water needs are met through drip irrigation. Planting within the high-tunnel system follows a three-year rotation of tomatoes, cut flowers, and warm season cover crops. A mixture of winter wheat (120 lb/acre sowing rate; Triticum aestivum) and crimson clover (20 lb/acre sowing rate; Trifolium
incarnatum) are grown during the fall and winter months and incorporated prior to bedding to meet the nutritional requirements for the crop.

**Transplant Production:** All transplants were produced in an air-inflated double-layer polyethylene greenhouse on the North Carolina State University campus (NCSU; Raleigh, NC). The rootstock cultivars Beaufort (De Ruiter, St. Louis, MO) and Shield (Rijk Zwaan, Salinas, CA) were chosen as these two have significantly different root system morphologies (Suchoff et al., 2017). The ‘Beaufort’ root system has a much thinner average diameter, higher specific root length, and shorter total root length than ‘Shield’. The indeterminate heirloom cultivar Cherokee Purple (CP; Johnny’s Selected Seeds, Winslow, ME) is what the grower traditionally grows and thus utilized as both the scion for the two rootstocks and the non-grafted control. Seeds were started in 72-cell plug trays (T.O. Plastics, Clearwater, MN) filled with an all-purpose potting mix (Fafard® 4P Mix; Sun Gro® Horticulture, Agawam, MA). To account for its slower germination time, ‘Beaufort’ was planted five days prior to both ‘Shield’ and CP scion material. The non-grafted CP were seeded 5 d after ‘Shield’ and scion CP to take into account the 5 d needed for graft healing.

Seedlings were grafted utilizing the Japanese tube-graft method (Rivard and Louws, 2006). Grafts were healed in transparent plastic storage bins (26.5 inches length × 16 inches height × 12.5 inches width; Sterilite®, Townsend, MA) where they received constant 100 μmol·m⁻²·s⁻¹ of photosynthetically active radiation (4 × 6500 °K Spectralux® T5 HO Fluorescent lamp; Sunlight Supply, Inc., Vancouver, WA) in an indoor, controlled environment maintained at 78.8 °F. High relative humidity (>95%) was maintained by placing ~1 inch of water at the bottom of the bin with the flats raised above the water level and the bin tops taped shut. Each bin held a single 72-cell plug tray and three bins fit under
each fluorescent lamp. Grafts were slowly acclimated to reduced humidity by gradually opening the tops until after 5 d tops were removed. Healed grafts were placed in a high-tunnel on NCSU campus for 3 d to harden off prior to transplanting.

Field design: A strip-till system was used to prepare beds one month prior to transplanting. An established winter wheat and crimson clover was mowed and three-foot wide beds were cultivated to a depth of 12 inches and then tilled to a depth of 6 inches. Compost was incorporated into each bed at a rate of 2,352 ft³/acre bed to supplement crop nutritional needs. Each high-tunnel covers four rows spaced 5 ft apart. To limit potential rainwater intrusion, the inner two rows of two conjoined high-tunnels were used in the study. Drip tape with emitters spaced 8 inches apart (0.94 gal/min per 100 ft, 8 Mil Lightweight 5/8 inch Aqua-Traxx®; The Toro Company, El Cajon, CA) was placed on top of the soil and 4 ft-wide black woven groundcover placed over each bed. An 8-inch deep trench was dug across the middle of each bed and a 0.75-inch thick wooden board was placed in the trench. This board reduced the lateral movement of water within the soil profile between the two sections. Drip tape at this midpoint was cut and the two sections were randomly assigned an irrigation treatment. The study was arranged in a split-plot design with the whole-plot arranged in a randomized complete block design with four blocks. Irrigation treatments (whole-plot) were either 100% the grower norm (3 h every other day; 14,741 gal/acre) or 50% (1.5 h every other day; 7,370 gal/acre). Each half block was randomly assigned an irrigation treatment. A dual line irrigation timer (Aquedue Duplo® Evolution; Claber®, Geneva, IL) allowed for independent irrigation of each half block at the appropriate time. Two headers (1.5 inch Blue Stripe® Oval Hosing; The Toro Company, El Cajon, CA) were connected to the timer, one for each irrigation treatment, and ran along the entrance of each high-tunnel. Drip lines were
connected to the appropriate header depending on the assigned irrigation treatment. Mainline tubing (1 inch Blue Stripe® Poly Tubing; The Toro Company, El Cajon, CA) was attached to the appropriate header and ran down the half of the block where it was connected to the cut drip tape at the midpoint. Total applied irrigation in 2016 was 434,816 gal/acre and 810,739 gal/acre for 50% and 100% irrigation treatments, respectively. In 2017, 383,240 gal/acre and 766,482 gal/acre was applied to the 50% and 100% irrigation treatments, respectively.

The three rootstock treatments (split-plot) were randomly assigned within each whole-plot. Split-plots comprised of 10 plants spaced 18 inches apart. Transplanting occurred on 22 Apr. 2016 and 14 Apr. 2017. In 2016 all transplants received 100% irrigation regime for the first week until established. Plants were pruned once two-weeks after transplant to remove suckers and vertically trained along a wire fence with tie ribbon once a week.

Data collection: Matric water potential sensors (MPS-6; METER Group, Pullman, WA) were placed randomly within each whole-plot replicate (n=8) at a depth of approximately 12 inches and soil matric water potential readings were taken every hour and stored in a data logger (EM50; METER Group).

Plant height was measured from the internal third, fifth, and seventh plants within a plot. In 2016, measurements started seven days after transplant (DAT) and were collected weekly until 42 DAT, at which point fruit started to develop. Plant height measurements in 2017 were collected in the same fashion but were extended until 70 DAT since height results from 2016 did not appear to plateau at 42 DAT.

Harvest began 59 DAT in 2016 and 63 DAT in 2017. Harvests occurred twice a week for 8 weeks in 2016 (n=16 harvests) and 6 weeks in 2017 (n=12 harvests). All fruit were harvested within a plot once they reached the turning stage (between 10% and 30% of the
fruit showing pink/red coloration; USDA, 2005). Fruit were graded based on grower standards: grade A fruit are those free of blemishes or disfigurement and are larger than 2 inches in diameter, grade B fruit are those with slight blemishes, disfigurement, but are still marketable, and culls are those fruit rendered unmarketable due to cracking, disease, or pest injury (Fig. 1). Fruit in each grade were counted and weighed. Total fruit weight was divided by total fruit count for each grade at the end of the season to give an average individual fruit weight for grades B and A. Proportions of total harvest based on grade were calculated at the end of the season. iWUE (lb/gal) was calculated for grade A fruit as well as all marketable fruit (grade A and grade B combined) by dividing the total yields at the end of the season by the total volume of water applied.

In 2017 potato aphid (*Macrosiphum euphorbiae*) infestation in the first 2 months of growth was significant enough to warrant application of a contact insecticide via foliar spray at a rate of 1.81oz/100 gal a.i. (Mycotrol® ESO; BioWorks®, Victor, NY). Application effectively managed the pest population for the remainder of the season.

At the end of each season, six random plants were uprooted and root systems checked for any disease or nodulation caused by root knot nematodes (*Meloidogyne sp.*); no nodulation or disease was observed.

*Data Analysis:* All data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute Inc. Cary, NC). Irrigation, rootstock, and year were analyzed as fixed effects with block and irrigation × block as random. Fruit count and proportions of total harvest based on grade were modeled using a negative binomial and beta distribution, respectively, with the canonical link functions. Pearson chi-squared statistics divided by the degrees of freedom (ϕ) were checked for over-dispersion and distribution goodness-of-fit. Cumulative
yield over time for grades A and B and plant height were analyzed as repeated measures with a heterogenous first-order autoregressive covariance structure. Since both height measurements and number of harvests differed by year, these data were analyzed separately by year. Residual plots were checked for potential outliers and heteroscedasticity. Tukey’s honest significant difference test was used to compare means when appropriate. Finally, we ran an economic assessment to compare the financial feasibility of grafting utilizing production values from the grower as well as those published by Rysin and Louws (2015).

Results

Matric water potential in the 50% irrigation treatment was more negative than the 100% treatment in both 2016 and 2017 (Fig. 2). In 2016, these differences became more pronounced midway through July and through the rest of the season. In 2017, these differences were observed earlier in the season, towards the end of May, and remained until the end of the study.

Plant height: The interaction of rootstock and DAT was significant in both 2016 and 2017 for plant height; slight differences (less than two inches) were observed early in the season, but these differences were not observed after 35 DAT (data not shown).

Yield: None of the first-order interactions with year nor the second order interaction were significant for any yield response variables (Table 1). Average fruit weight for grades A and B were not affected by any treatments in this study. Total weight and count for culls and grade B fruit were different between years; values for both grades were significantly higher in 2017 than in 2016 (Fig. 3). The proportion of total fruit weight composed of grade A and culls was affected by the main effects of rootstock, irrigation, and year (Table 1). S and CP had a higher proportion of their total fruit weight composed of culls compared to B whereas
the latter had a higher proportion of grade A fruit compared to S and CP (Fig. 4). The 100% irrigation treatment resulted in a higher proportion of grade A fruit and concurrent reduction in proportion culls compared to the 50% treatment (Fig. 4). Finally, 2016 had a higher proportion of grade A fruit and less culls than 2017 (Fig. 4).

The rootstock × irrigation interaction was significant for grade A count and weight as well as marketable fruit count and weight (Table 1). At 100% irrigation there was no difference in grade A fruit count among rootstock treatments (Fig. 5C); however, grade A fruit weight was higher in B (33,585.5 lb/acre) compared to CP (24,184.5 lb/acre; Fig. 5A). No differences were observed in marketable fruit count or weight among rootstock treatments at 100% irrigation (Fig. 5B and 5D). At 50% irrigation, grade A fruit count was higher in B (55,612 fruit/acre) than both S (33,614 fruit/acre) and CP (31,581 fruit/acre; Fig. 5C). Consequently, grade A fruit weight followed the same trend with B yields (36,424.9 lb/acre) being significantly higher than S (21,648.6 lb/acre) and CP (19,388.6 lb/acre; Fig. 5A). These trends were also observed in marketable fruit weight and count; at 100% irrigation no differences were observed among the rootstock treatments, but at 50% B yielded significantly higher fruit count and weight (Fig. 5B and 5D). Furthermore, B yielded higher grade A and marketable fruit weight and count at 50% irrigation compared to CP at 100% irrigation (Fig. 5A-5D). In S and CP there was a trend towards reduced fruit count and weight with a reduction in irrigation; however, this trend was not observed in B.

The longitudinal analysis of cumulative yield resulted in a significant rootstock × harvest interaction for grade A fruit production in 2016 and 2017 (Table 2). In 2016, no differences were observed among the rootstock treatments over the first 8 harvests; however, starting at harvest 9 until the last harvest, B yielded significantly more than CP with S
intermediate of the two (Fig. 6). In 2017, differences were observed earlier in the harvest. Starting at harvest 3 B consistently yielded more than both CP and S.

iWUE for both grade A and marketable fruit was affected by the rootstock × irrigation interaction (Table 1). At 100% irrigation there were no differences in iWUE among the rootstock treatments for grade A or marketable fruit (Fig. 7A and 7B). iWUE was higher for the 50% irrigation treatment than the 100% treatment. At the 50% irrigation treatment B had significantly higher iWUE for both grades compared to S and CP. iWUE for B at 50% irrigation, regardless of fruit grade, was nearly three times higher than CP at the 100% irrigation treatment. The main effect of year significantly affected marketable fruit iWUE (Table 1); 2017 had an overall higher iWUE compared to 2016.

Economic assessment: Using the values from Rysin and Louws (2015) of $0.12 and $1.02 per non-grafted and grafted transplant, respectively, an additional $5,227.2 per acre would need to be invested for the utilization of grafted plants (Table 3). Furthermore, due to the significantly higher grade A fruit production of B even at 50% irrigation, harvest labor would increase by $1,713.65 per acre. Even with these additional investments, net revenue would increase by $35,900.41 per acre when utilizing B at 50% irrigation compared to CP at 100% irrigation, amounting to an 44.6% increase in net revenue.

Discussion

While the initial implementation of grafted tomatoes was for the management of soilborne pathogens (Kubota et al., 2008), additional rootstock-derived benefits exist outside of disease resistance (Djidonou et al., 2013a; Ntatsi et al., 2017; Schwarz et al., 2010). Here we show that in the absence of disease pressure, grafting onto ‘Beaufort’ rootstock significantly improved iWUE and yields at 50% the grower normal irrigation rate (Fig. 5A-
D, Fig. 7A-B). These results are in agreement with Djidonou et al. (2013a) who found that grafting the determinate cultivar Florida-47 onto Beaufort improved iWUE in open-field production. In their study, the authors demonstrated improved iWUE regardless of irrigation regime. We only observed iWUE differences at the 50% irrigation regime. The discrepancies between studies may be due to the fact that our study utilized an indeterminate tomato cultivar and conducted the study under protection from rain. The authors also found that grafting onto ‘Multifort’ rootstock improved iWUE. Both ‘Multifort’ and ‘Beaufort’ are interspecific hybrids \( (S. lycopersicum \times S. habrochaites) \) and were shown to have similar root system morphology (Suchoff et al., 2017). In our study, ‘Shield’ was chosen as its root system is composed of much thicker, longer roots, with a lower specific root length compared to ‘Beaufort’ (Suchoff et al., 2017). Crops with thin average root diameter and high specific root length show an increase in hydraulic conductivity (Huang and Eissenstat, 2000). Furthermore, high specific root length (SRL) allows for root system development with minimized photosynthate investment compared to root systems with low SRL (Eissenstat, 1992). The improved yield and iWUE observed here and by Djidonou et al. (2013a) may be due to a thinner root system in ‘Beaufort’ that can more easily absorb and utilize available water and, due to high SRL, can increase root soil exploration with minimal photosynthate investment.

Differences in plant height were observed among rootstocks in both years of the study; however, no overall trend was seen nor do these differences explain yield or iWUE differences among rootstocks. Khah et al. (2006) found no differences in plant height among grafted tomatoes on different rootstocks compared to non-grafted and self-grafted controls when grown in greenhouse conditions; however, when grown in open-field conditions,
differences were observed. The more favorable environment experienced by plants grown in protected culture may reduce the plant height-diminishing stresses compared to open-field production.

Higher cull and grade B fruit were produced in 2017 compared to 2016 (Fig. 3A-D). These yearly differences are most likely due to the potato aphid infestation that occurred early in 2017; prior work has demonstrated a reduction in tomato fruit quality due to potato aphid infestation (Walgenbach, 1997). We observed a higher amount of damage and disfigurement early in the 2017 harvest season which coincided with potato aphid infestation and the amount of this damaged fruit reduced later in the season following insecticide application.

Grafting onto ‘Beaufort’ increased the proportion of grade A fruit produced (Fig. 4). Di Gioia et al. (2010) and Leonardi and Giuffrida (2006) found that ‘Beaufort’ increased marketable fruit production in conditions lacking any significant disease pressure. This increase in marketable fruit production may be due to improved nutrient uptake when using ‘Beaufort’ as a rootstock (Leonardi and Giuffrida, 2006).

The use of grafted tomatoes requires a high initial investment. In situations where disease pressure is significant, utilizing grafted tomatoes with resistant rootstocks has the potential to offset that initial investment; however, net returns are dependent on disease pressure and market prices (Djidonou et al., 2013b; Rysin and Louws, 2015). Here we show that grafting ‘Cherokee Purple’ onto ‘Beaufort’ increased net revenue by 44.6% while reducing the water applied by 50% (Table 3). This reduction amounts to a saving of 375,923 gal/acre and 383,242 gal/acre in 2016 and 2017, respectively. Irrigation for this farm comes from an on-site pond and, as such, water is not an annually limited resource. In areas where
water is less plentiful and growers must pay for irrigation water, such as California, the increased iWUE when utilizing ‘Beaufort’ as a rootstock has the potential to further reduce initial transplant investment costs by decreasing irrigation expenses.

Conclusion

This study is one of the first to demonstrate the ability of specific tomato rootstocks to improve iWUE in an on-farm situation. With the addition of improved iWUE, rootstock-scion combinations can be selected to meet market demand, disease pressure, and limited water availability. This improved iWUE may be due to differences in root system morphology (Suchoff et al., 2017). Future work is needed to determine the specific morphological or physiological aspects of the ‘Beaufort’ rootstock root system that improve water use efficiency under limiting conditions. This information will allow for further rootstock selection and aid in our understanding of plant-water relations.
Literature Cited:


Table 1. Three-way analysis of variance for tomato yield data and water use efficiency for high-tunnel grown grafted tomatoes.

<table>
<thead>
<tr>
<th></th>
<th>Grade A</th>
<th></th>
<th>Grade B</th>
<th></th>
<th>Marketable</th>
<th></th>
<th>Cull</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual</td>
<td>Total</td>
<td>Individual</td>
<td>Total</td>
<td>Individual</td>
<td>Total</td>
<td>Individual</td>
<td>Total</td>
</tr>
<tr>
<td><strong>Effect</strong></td>
<td>df</td>
<td>wt</td>
<td>Count</td>
<td>wt</td>
<td>Proportion</td>
<td>wt</td>
<td>Count</td>
<td>wt</td>
</tr>
<tr>
<td>Rootstock (R)</td>
<td>2</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irrigation (I)</td>
<td>1</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>R x I</td>
<td>2</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Year (Y)</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Y = R</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Y x I</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Y x R x I</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at $P \leq 0.05$, significant at $P \leq 0.05, 0.01, 0.001$, respectively.

$^2$Fruit grades based on grower specifications. Grade A fruit are those with no blemishes or disfigurement larger than 2 inches in diameter. Grade B fruit are those with slight disfigurement, but still marketable, and culls are any non-marketable fruit. Marketable fruit is the combination of grades A and B.

$^3$Proportion of total fruit weight by grade.

$^4$Irrigation water use efficiency for grade A and marketable fruit calculated as the total weight of fruit divided by the volume of water applied for the season.
Table 2. Longitudinal analysis of cumulative tomato yield of grades A and B fruit from high-tunnel grown grafted tomatoes for 2016 and 2017.

<table>
<thead>
<tr>
<th>Effect</th>
<th>2016†</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Grade B wt</td>
</tr>
<tr>
<td>Rootstock ©</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Irrigation (I)</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>R × I</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Harvest‡ (H)</td>
<td>11</td>
<td>***</td>
</tr>
<tr>
<td>R × H</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>I × H</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>R × I × H</td>
<td>22</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at $P \leq 0.05$, significant at $P \leq 0.05$, 0.01, 0.001, respectively.

†Harvests occurred twice a week for 8 weeks and 6 weeks in 2016 and 2017, respectively.

‡Years were analyzed separately due to different lengths of data collection for harvests.

³Fruit grades based on grower specifications. Grade A fruit are those with no blemishes or disfigurement large than 2 inches in diameter. Grade B fruit are those with slight disfigurement, but still marketable, and culls are any non-marketable fruit.

1 inch = 2.54 cm.
Table 3. Economic assessment of high-tunnel grown non-grafted ‘Cherokee Purple’ at full irrigation versus grafted onto ‘Beaufort’ rootstock at reduced irrigation.

<table>
<thead>
<tr>
<th></th>
<th>‘Cherokee Purple’ Non-grafted 100% Irrigation</th>
<th>‘Cherokee Purple’ grafted onto ‘Beaufort’ 50% Irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of transplants (plants/acre)</td>
<td>5,808</td>
<td>5,808</td>
</tr>
<tr>
<td>Price of transplant ($/plant)(^z)</td>
<td>0.12</td>
<td>1.02</td>
</tr>
<tr>
<td>Cost of transplants ($/acre)</td>
<td>696.96</td>
<td>5,924.16</td>
</tr>
<tr>
<td>Average yield of grade A (lb/acre)</td>
<td>24,184.51</td>
<td>36,424.87</td>
</tr>
<tr>
<td>Labor cost ($/lb)(^y)</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Harvest cost ($/acre)</td>
<td>3,385.83</td>
<td>5,099.48</td>
</tr>
<tr>
<td>Sale price ($/lb)</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Gross revenue ($/acre)</td>
<td>84,645.79</td>
<td>127,487.05</td>
</tr>
<tr>
<td>Net revenue ($/acre)</td>
<td>80,563.00</td>
<td>116,463.41</td>
</tr>
</tbody>
</table>

\(^z\)Price of transplants derived from Rysin and Louws (2015).

\(^y\)Values for labor and sales price specified by the grower.
Figure 1. Grade A (bottom row) and Grade B (top row) fruit based on grower specifications.
Figure 2. Matric water potential of high-tunnel soils for two irrigation treatments of either 100% or 50% the grower norm at a depth of 12 inches for 2016 and 2017.

1 kPa = 0.01 bar.

1 inch = 2.54 cm.
Figure 3. Total cull fruit weight (A) and count (C) and grade B fruit weight (B) and count (D), ± standard error from high-tunnel grown grafted tomatoes for 2016 and 2017. Means with common letters within grade count or weight are not different (Tukeys HSD; α = 0.05) and represent the average of three rootstock treatments, two irrigation treatments, and four replicate samples (n=24 data points for each mean). Grade B fruit are those with slight disfigurement but remain marketable, cull fruit are those that are rendered unmarketable due to crack or pest damage or have a diameter less than 2 inches.

1 inch = 2.54 cm.

1 lb = 0.4536 kg.

1 lb/acre = 1.12 kg·ha⁻¹.

1 fruit/acre = 2.47 fruit/ha.
Figure 4. Rootstock, irrigation, and year main effects on fruit grade as a proportion of total fruit weight for high-tunnel grown grafted tomatoes. Means with common letters within the same grade and main effect are not different (Tukey's HSD; α = 0.05) and represent the average of two irrigation treatments, four replicates and two repeated experiments for rootstock treatment effect (n=16 data points for each mean), three rootstock treatments, four replicates, and two repeated experiments for irrigation treatment effect (n=24 data points for each mean), and three rootstock treatments, two irrigation treatments, and four replicates for year main effect (n=24 data points for each mean). Tomato cultivar Cherokee Purple was grafted onto either Beaufort or Shield rootstocks or left non-grafted (NG) as a control. Grade A fruit are those free of blemishes or disfigurement and are larger than 2 inches in diameter, grade B are fruit with slight disfigurement but remain marketable, and cull fruit are those not marketable due to cracking or pest damage or have a diameter less than 2 inches. 1 inch = 2.54 cm.
Figure 5. Total grade A fruit weight (A) and count (C) and marketable fruit weight (B) and count (D), ± standard error from high-tunnel grown grafted tomatoes receiving 100% or 50% of grower normal irrigation rate. Means with common letters within a grade are not different (Tukey’s HSD; α = 0.05) and represent the average of four replicates and two repeated experiments (n=8 data points for each mean). Tomato cultivar Cherokee Purple was grafted onto either Beaufort or Shield rootstocks or left non-grafted (NG) as a control. Grade A fruit are those free of blemishes or disfigurement and are larger than 2 inches in diameter. Marketable fruit are the combination of grade A and grade B fruit, the latter being fruit with slight disfigurement but remain marketable.

1 inch = 2.54 cm.

1 lb = 0.4536 kg.

1 lb/acre = 1.12 kg·ha⁻¹.

1 fruit/acre = 2.47 fruit/ha.
Figure 7. Irrigation water use efficiency for grade A (A) and marketable fruit (B), ± standard error from high-tunnel grown grafted tomatoes receiving 100% or 50% of grower normal irrigation rate. Means with common letters within a grade are not different (Tukeys HSD; α = f0.05) and represent the average of four replicates and two repeated experiments (n=8 data points for each mean). Tomato cultivar Cherokee Purple was grafted onto either Beaufort’ or Shield rootstocks or left non-grafted (NG) as a control. Grade A fruit represent those fruit free of blemishes or disfigurement and are larger than 2 inches in diameter. Marketable fruit are the combination of grade A and grade B fruit, the latter being fruit with slight disfigurement but remain marketable.

1 inch = 2.54 cm.

1 lb = 0.4536 kg.

1 lb/gal = 0.1198 kg·L⁻¹.
Figure 6. Longitudinal analysis of cumulative yield of grade A fruit from high-tunnel grown grafted tomatoes in 2016 and 2017. Means and common letters within a grade are not different (Tukey’s HSD; α = 0.05) and represent the average of four replicates and two irrigation treatments (n=8 data points for each mean). Tomato cultivar Cherokee Purple was grafted onto either Beaufort or Shield rootstocks or left non-grafted (NG) as a control. Grade A fruit are those free of blemishes or disfigurement and are larger than 2 inches in diameter.
CHAPTER 3

Grafted Tomato Shoot and Root Responses to Drying Soils
Grafted Tomato Shoot and Root Responses to Drying Soils

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S Department of Agriculture, under award number 2016-51181-25404.

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Subject Category: Environmental Stress Physiology

Grafted Tomato Shoot and Root Responses to Drying Soils

*Additional index words.* Rootstock; Herbaceous Grafts; Irrigation; Heirloom tomato; root system morphology.

*Summary.* Improvement of crop water use is imperative. How plants respond to limited water can dictate their ability to better utilize 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, and net CO₂ assimilation rate was observed with scions grafted on ‘Beaufort’. In addition, this rootstock had a significantly longer total root system (118.6 m) compared to ‘Shield’ (94.9 m) and the self-grafted control (104.2 m). Furthermore, 76.40% of the total root length observed in ‘Beaufort’ was composed of very thin diameter roots (\(\Phi < 0.5 \text{ mm}\)), which was higher than ‘Shield’ (73.67%) and the self-grafted control (69.07%). The only significant rootstock × irrigation interaction observed was for effective quantum yield of photosystem II (\(\phi_{\text{PSII}}\)). At normal irrigation there were no differences among the
rootstock treatments; however, at reduced irrigation ‘Beaufort’ had significantly higher $\phi_{\text{PSII}}$ 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 utilizing ‘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 utilize approximately 70% of the available water but to meet the demands of a growing global population, water consumption will need to increase by 70% - 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.

How a plant responds and adapts 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; Hsaio, 1973; Shao et al., 2008). These responses can be grouped into either dehydration tolerance and/or dehydration avoidance based on their plant 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 (*Craterostigma plantagineum*). Developing more resilient food crops that utilize 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 stomatal conductance leads to a concomitant reduction in CO₂ diffusion and consequent reduction in photosynthesis and carbon assimilation (Chaves, 1991; Hsaio, 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 select 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). Certain rootstocks have demonstrated the ability to improve abiotic stress tolerance, including limited water, in susceptible scions (Djidonou et al., 2013; Schwarz et al., 2010). 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 or not these rootstocks respond differently to drying soils at a root system morphological level is unknown. As such, the objectives of the following study 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**

**Location.** Trials were conducted in an air-inflated double-layer polyethylene greenhouse on North Carolina State University’s Horticultural Field Laboratory during the months of July and August. Temperatures were maintained (30 ± 3 °C day / 25 ± 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., in review). 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). Due to the slow germination of B, it was seeded three days prior to 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). Grafts were healed in transparent plastic storage bins (67.3 cm length × 40.6 cm height × 31.8 cm width; Sterilite®, Townsend, MA) where they received 100 μmol·m⁻²·s⁻¹ of photosynthetically active radiation (4 × 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 five days 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 two days.

Pot preparation. Black polyethylene pots with a volume of 7.33 L and dimensions of 24.1 cm (top diameter) x 20.3 cm (height) x 20.3 cm (bottom diameter) (Poly-Tainer #3 Short; Hummert International, Earth City, MO) were lined with woven 20 × 20 mesh of 0.02 cm diameter thread (~0.016 cm² opening size; Clear Advantage Charcoal Fiber Glass Insect Screen, New York Wire, 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 The 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 (~15 min.) they were weighed to determine individual container capacity values (White and Mastalerz, 1966).

Experimental set-up. 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 × 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 five-day period. All pots were weighed at 8 a.m. and the volume of water lost due to transpiration was determined 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 terminated. The first trial of the study started on 6 July, 2017 and terminated 13 July, and then repeated 11 Aug. till 18 Aug. 2017.

Data collection. Prior to 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-, 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 taken at 8 a.m. and 5 p.m. and recorded.

Leaf gas exchange and fluorescence measurements were taken the day prior to 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 CO₂ assimilation (A, µmol·m⁻²·s⁻¹), stomatal conductance (gs, mmol·m⁻²·s⁻¹), and effective quantum yield of photosystem II (φPSII) were measured between 10 a.m. and 1 p.m. Photosynthetic photon flux
density within the chamber was set to 1400 µmol·m⁻²·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 utilizing 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:

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

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

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⁻¹ neutral red dye solution (Sigma Aldrich Co., St. Louis, MO) for 24 h at 6.7 °C. The dying process improved resolution during scanning and image acquisition (Bouma et al., 2000). Roots were rinsed, placed in a 30 x 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 utilized, 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 diameter class length; RDCL). Roots were dried at 70 °C for 24 h (Thelco 130D Laboratory Oven, Precision Scientific Co.) 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).

Data 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, ϕPSII, relative water contents) were modeled using a beta distribution and canonical link function. Pearson chi-squared statistics divided by the degrees of freedom (ϕ) 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 was back-transformed to its 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 (Fig. 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 (Fig. 1-2).

*Scion morphology and physiology.* The main effect of irrigation affected all scion morphological and physiological responses measured except for $\phi_{\text{PSII}}$ 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 the 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 in $g_s$ and $A$; B had higher levels of both responses compared to S and C but no difference between the latter two (Fig. 6). At normal irrigation, $\phi_{\text{PSII}}$ was similar among the three rootstock treatments; however, at reduced irrigation B had higher $\phi_{\text{PSII}}$ (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 not affected by irrigation treatments (Table 2); however, since total root length decreased with reduced 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 to normal irrigation treatments (Table 4). This increase is due to the concurrent reduction in shoot dry weight and increase in root dry weight (Table 4). Shoot dry weight was not affected by rootstock treatments; however, root dry weight was significantly higher in C compared to 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 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 U.N. 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 list 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 thus normally does not experience 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 CO₂ assimilation, and stomatal conductance regardless of irrigation regime (Figs. 5 and 6A-B). The absence of significant rootstock × irrigation interactions for all responses other than \( \phi_{\text{PSII}} \), and presence of significant rootstock main effects is 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’ are in agreement with the findings of Atunlu 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 to both S and C (Fig. 7A and C). Both B and S had significantly thinner average root diameters compared to 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 to 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 to C and S, respectively (Table 3).

Accordingly, the ability of B to maintain higher relative water content, net CO2 assimilation, and stomatal conductance 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 non-grafted ‘Florida 47’ regardless of irrigation treatments and consequently improved irrigation water use efficiency. Suchoff et al. (in review) 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 non-grafted ‘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 to a non-grafted 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 note that this rootstock showed a general negative effect on plant growth and reduced stomatal conductance compared to the other treatments. Similarly, we found that S tended to reduce leaf area compared to our control (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 stomatal conductance 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$_2$, 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 utilizes 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 (Fig. 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 approximately 25% longer than S (116.8 m vs. 94.9 m, respectively; Fig. 7A), 
and a higher proportion of B’s root length 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 utilize 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 as it relates to water availability. Water, or the lack 
thereof, is a growing challenge faced by all involved in agriculture and food production. It is 
critical 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., in 
review), 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 as ‘Beaufort’, as well as investigating whether unique molecular or
biochemical changes are associated with different rootstocks when grown in conditions of limited water.
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Table 1. Results of analysis of variance for the impact of rootstock and irrigation on greenhouse grown tomato scion morphology and physiology.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Leaf Dry wt</th>
<th>Stem Dry wt</th>
<th>Shoot Dry wt</th>
<th>Leaf RWC(^y)</th>
<th>Stem RWC</th>
<th>Leaf Area</th>
<th>Specific Leaf Area(^x)</th>
<th>g(_w)</th>
<th>A(^v)</th>
<th>(\phi_{PSII})(^u)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootstock</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>***</td>
<td>**</td>
<td></td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Irrigation</td>
<td>1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Rootstock (\times) Irrigation</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS **</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at \(P \leq 0.05\), significant at \(P \leq 0.05, 0.01, 0.001\), respectively.

\(^{z}\)Shoot dry weight is the combination of leaf and stem dry weights.

\(^{y}\)Relative water content.

\(^{x}\)Calculated as leaf area divided by leaf dry weight.

\(^{w}\)Stomatal conductance.

\(^{v}\)Net CO\(_2\) assimilation rate.

\(^{u}\)Efficiency of photosystem II, calculated as the proportion of photons used by photosystem II for photochemistry.
Table 2. Results of analysis of variance for the impact of rootstock and irrigation on greenhouse grown tomato root systems.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Root Dry wt</th>
<th>Root:Shoot$^z$</th>
<th>Average Root Diameter</th>
<th>Total Root Length</th>
<th>Specific Root Length$^y$</th>
<th>DCL1$^x$</th>
<th>DCL2</th>
<th>DCL3</th>
<th>RDCL1$^w$</th>
<th>RDCL2</th>
<th>RDCL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootstock</td>
<td>2</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Irrigation</td>
<td>1</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Rootstock × Irrigation</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at $P \leq 0.05$, significant at $P \leq 0.05$, 0.01, 0.001, respectively.

$^z$Ratio of root dry weight to shoot dry weight.

$^y$Calculated as total root length divided by root dry weight.

$^x$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.

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

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>DCL1 (cm)</th>
<th>DCL2 (cm)</th>
<th>DCL3 (cm)</th>
<th>RDCL1&lt;sup&gt;y&lt;/sup&gt;</th>
<th>RDCL2</th>
<th>RDCL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Cherokee Purple’</td>
<td>7214.2 b</td>
<td>2496.0 a</td>
<td>708.2 a</td>
<td>0.6907 e&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.2399 a</td>
<td>0.0678 a</td>
</tr>
<tr>
<td>Self-grafted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Beaufort’</td>
<td>9069.3 a</td>
<td>2245.2 b</td>
<td>542.7 b</td>
<td>0.7640 a</td>
<td>0.1893 c</td>
<td>0.0458 c</td>
</tr>
<tr>
<td>‘Shield’</td>
<td>7018.8 b</td>
<td>1954.1 c</td>
<td>511.5 b</td>
<td>0.7367 b</td>
<td>0.2078 b</td>
<td>0.0543 b</td>
</tr>
<tr>
<td>Irrigation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8154.0 a</td>
<td>2229.8</td>
<td>622.9 a</td>
<td>0.7414 a</td>
<td>0.1832 b</td>
<td>0.0552</td>
</tr>
<tr>
<td>Reduced</td>
<td>7380.8 b</td>
<td>2233.6</td>
<td>551.9 b</td>
<td>0.7214 b</td>
<td>0.2861 a</td>
<td>0.0554</td>
</tr>
</tbody>
</table>

<sup>x</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 (Tukeys HSD; α = 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 weigh, shoot dry weight, and root:shoot ratio for greenhouse grown tomatoes.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Root Dry wt (g)</th>
<th>Shoot Dry wt(^{\text{y}}) (g)</th>
<th>Root:shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Cherokee Purple’ Self-grafted</td>
<td>1.086 a(^{2})</td>
<td>4.295</td>
<td>0.258 a</td>
</tr>
<tr>
<td>‘Beaufort’</td>
<td>0.969 ab</td>
<td>4.648</td>
<td>0.215 b</td>
</tr>
<tr>
<td>‘Shield’</td>
<td>0.892 b</td>
<td>4.113</td>
<td>0.220 b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Root Dry wt (g)</th>
<th>Shoot Dry wt(^{\text{y}}) (g)</th>
<th>Root:shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.938 b</td>
<td>5.115 a</td>
<td>0.183 b</td>
</tr>
<tr>
<td>Reduced</td>
<td>1.027 a</td>
<td>3.589 b</td>
<td>0.286 a</td>
</tr>
</tbody>
</table>

\(^{2}\)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).

\(^{\text{y}}\)Shoot dry weight is the combination of stem and leaf dry weights.
Figure 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 a.m. and 5 p.m. and water added to only the normal irrigation treatment pots to maintain pot weight at container capacity.
Figure 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).
Figure 3. Main effect of irrigation on leaf dry weight (A), stem dry weight (B), and stem relative water content (C) ± standard error. Means with common letters within a response are not different (Tukeys HSD; α = 0.05) and represent the average of two trials, five blocks, and three rootstock treatments (n=30 data points for each main effect marginal mean).
Figure 4. Main effect of rootstock and irrigation on total leaf area (A) and specific leaf area (B) ± standard error. Means with common letters within a response are not different (Tukeys 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’ rootstock. *** denotes a significant difference between irrigation treatment means (Tukeys HSD; $\alpha = 0.05$) which represent the average of two trials, five blocks, and three rootstock treatments ($n=30$ data points for each mean).
Figure 5. Main effect of rootstock on leaf relative water content ± standard error. Means with common letters are not different (Tukeys HSD; α = 0.05) and represent the average of two trials, five blocks, and two irrigation treatments (n=20 data points for each marginal mean). Rootstock treatments include self-grafted ‘Cherokee Purple’ tomato (C), ‘Cherokee Purple’ grafted onto ‘Beaufort’ rootstock (B), and ‘Cherokee Purple’ grafted onto ‘Shield’ rootstock. *** denotes a significant difference between irrigation treatment means (Tukeys HSD; α = 0.05) which represent the average of two trials, five blocks, and three rootstock treatments (n=30 data points for each marginal mean).
Figure 6. Main effect of rootstock on net CO$_2$ assimilation (A), stomatal conductance (B), and the interaction of rootstock and irrigation on effective quantum yield of photosystem II (C). ± standard error. Means for net CO$_2$ assimilation and stomatal conductance with common letters are not different (Tukeys 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’ rootstock. *** denotes a significant difference between irrigation treatment means (Tukeys 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).
Figure 7. Main effect of rootstock on total root length (A), average root diameter (B), and specific root length (C) ± standard error. Means with common letters within a response are not different (Tukey's HSD; α = 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’ rootstock. *** denotes a significant difference between irrigation treatment means (Tukey's HSD; α = 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).
CHAPTER 4

Improving Tomato Cold Tolerance through Grafting
Improving Tomato Cold Tolerance through Grafting

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S Department of Agriculture, under award number 2016-51181-25404.

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Subject Category: Environmental Stress Physiology

Improving Tomato Cold Tolerance through Grafting

*Additional index words.* Rootstock; Herbaceous Grafts; ‘Multifort; ‘Shield’; ‘Moneymaker’; root system morphology; suboptimal temperature; *Solanum habrochaites*.

*Summary.* Tomatoes (*Solanum lycopersicum*) are a warm-season, cold-sensitive crop that show slower growth and development with temperatures below 18 °C. Improving suboptimal temperature tolerance would allow for earlier planting of field-grown tomatoes as well as a reduction in energy inputs for heating greenhouses. Grafting tomatoes onto high-altitude wild *Solanum habrochaites* accessions has proven effective at improving scion suboptimal temperature tolerance in limited experiments. The following study was conducted to determine whether commercially available tomato rootstocks with differing parental backgrounds and root system morphologies can improve the tolerance of scion plants to suboptimal temperature. Two controlled environment growth chambers were utilized and maintained at either optimal (25 °C day / 20 °C night) or suboptimal (15 °C day / 15 °C night) temperatures. The cold-sensitive tomato cultivar Moneymaker was used as the non-grafted and self-grafted control as well as scion on ‘Multifort’, ‘Shield’, and *S. habrochaites* LA 1777 rootstocks. ‘Multifort’ rootstock significantly reduced the amount of cold-induced leaf area reduction and maintained higher levels of CO₂ assimilation and photosystem II quantum efficiency. ‘Multifort’ maintained significantly longer roots, having 42% to 56% more fine root (diameter less than 0.5 mm) length compared to the other rootstock treatments. Leaf starch concentration was significantly lower in ‘Multifort’-grafted plants at suboptimal temperatures. The ability for ‘Multifort’ to maintain root growth at suboptimal temperatures may improve root system sink strength, thus allowing for proper movement of
photosynthate from leaf to root even under cold conditions. The results of this work demonstrate that commercially available rootstocks can be selected to improve suboptimal temperature tolerance in cold-sensitive scions at early stages of plant development.

Modern commercial tomato (*Solanum lycopersicum*) varieties originate from sub-tropical regions of South America. As such, warm temperatures between 18 °C and 25 °C are required for optimal growth and fruit production (Criddle et al., 1997; Ntatsi et al., 2017; Van Der Ploeg and Heuvelink, 2005). When exposed to temperatures below this optimal range, but above freezing, tomatoes show a marked decline in growth and yield. This reduction in growth at suboptimal temperatures is due to numerous cellular, biochemical, and physiological changes.

Photosynthesis is one of the most sensitive metabolic activities affected, both directly and indirectly, by suboptimal temperatures. Nearly all major components of photosynthesis, including photosystems I and II, the electron transport chain, and Rubisco show a decline in efficiency with cold stress (Allen and Ort, 2001; Kingston-Smith et al., 1997; Lynch, 1990). This is further exacerbated by a reduction in the amount of assimilable CO₂ caused by cold-induced stomatal closure (Allen and Ort, 2001). In response to cold soils, root system growth and carbon sink strength is reduced, resulting in a slowing of photosynthate export from the leaves and concurrent increase in leaf soluble sugar and starch concentrations (Ainsworth and Bush, 2011; Ntatsi et al., 2014; Rosa et al., 2004). While accumulated soluble sugars can have a protective effect in cold-stressed plant tissue (Gupta and Kaur, 2005), increased leaf-sucrose and starch concentrations lead to a downregulation of photosynthetic activity (Chiou and Bush, 1998; Goldschmidt and Huber, 1992; Paul and Foyer, 2001).
To limit cold-stress induced developmental growth retardation and yield loss, tomatoes produced in temperate regions are traditionally grown during the summer months or in fossil fuel heated greenhouses. The prospect of improved tolerance to suboptimal temperatures in tomatoes would lead to reduced greenhouse heating and consequent reduction in CO₂ generation from burning of fossil fuels. Furthermore, cold-tolerant tomato varieties could be planted earlier in the season, when soils are normally too cold, allowing growers to meet more lucrative early-season markets.

High-altitude wild tomato relatives exist that are more tolerant to suboptimal temperatures than cultivated *S. lycopersicum* (Venema et al., 1999). Unfortunately, breeding inter-specific hybrids that maintain elite fruit qualities while also tolerant to sub-optimal temperatures has not proven effective (Schwarz et al., 2010; Venema et al., 2005).

One means to bypass these breeding difficulties is through grafting; cold-sensitive elite tomato lines can be grafted onto cold-tolerant rootstocks. The high-altitude wild tomato relative *Solanum habrochaites* is known to be more tolerant of suboptimal temperatures (Venema et al., 1999, 2005) and has been the subject of numerous grafting studies. Venema et al. (2008) found that grafting the cold-sensitive *S. lycopersicum* cultivar Moneymaker onto *S. habrochaites* line accession LA 1777 (LA1777) improved shoot growth rate and total leaf area compared to self-grafted ‘Moneymaker’ when grown at 15 °C root zone temperature. The authors attribute this improved cold tolerance to the ability of LA1777 to maintain strong root growth as seen in an increased root:shoot dry-weight ratio, allowing for improved water and nutrient absorption even at low temperatures. A reduction in root hydraulic conductance is a common stress response to sub-optimal temperatures (Equiza et al., 2001; Fennell and Markhart III, 1997). Roots of *S. lycopersicum* and *S. habrochaites* showed this decrease in
hydraulic conductance at lower temperatures, but these values did not differ between species (Bloom et al. 2004). The results observed by Venema et al. (2008) may be attributed to LA1777’s ability to maintain higher water absorption by increasing root growth as a means to compensate for reduced hydraulic conductance.

Root system morphology plays a critical role in hydraulic conductance. Plants with thin average root diameter show marked increased in root hydraulic conductivity (Ho et al., 2005; Huang and Eissenstat, 2000; Rieger and Litvin, 1999). Unfortunately, little is known about the root system morphology of *S. habrochaites*. Furthermore, numerous commercially available interspecific tomato rootstocks (*S. lycopersicum × S. habrochaites*) exist but have not been investigated for cold tolerance. In order to address these research gaps the following study was conducted to: 1) compare shoot growth, photosynthetic activity, and leaf photosynthate concentrations of a cold-sensitive tomato cultivar when grafted onto an intraspecific tomato hybrid (*S. lycopersicum*) rootstock, an interspecific tomato hybrid (*S. lycopersicum × S. habrochaites*), and wild accession (*S. habrochaites*) at optimal and suboptimal temperatures and; 2) determine if differences exist among the rootstock root system morphologies at different temperatures.

*Materials and Methods*

*Location.* This study was conducted in two built-in controlled environment growth chambers (2.4 m wide × 3.7 m depth × 2.1 m height) in the North Carolina State University Phytotron (Raleigh, NC). Chambers were lit with a combination of T-5 fluorescent and incandescent bulbs so that plants received approximately 500 μmol-m⁻²⋅s⁻¹ of photosynthetically active radiation (PAR). Chamber temperatures were designated as optimal (25 °C day / 20 °C night) and suboptimal (15 °C day / 15 °C night). Relative humidity in
both chambers was maintained at 70% and ambient CO$_2$ concentrations of 400 parts per million (ppm).

Plant material. The indeterminate tomato cv Moneymaker (West Coast Seeds, B.C., Canada) was used due to its published sensitivity to cold (Ntatsi et al., 2014, 2017). Two commercially available rootstocks [‘Multifort’ (De Ruiter, St. Louis, MO) and ‘Shield’ (Rijk Zwaan, Salinas, CA)] were used as these two had significantly different root system morphologies (Surchoff et al., 2017). While both rootstocks are hybrids, ‘Multifort’ is interspecific (S. lycopersicum × S. habrochaites) whereas ‘Shield’ is intraspecific (S. lycopersicum). Additionally, the wild tomato species S. habrochaites line accession LA 1777 (LA 1777; C.M. Rick Tomato Genetics Resource Center, UC Davis, CA, United States) was used due to its documented cold tolerance (Ntatsi et al., 2014, 2017; Venema et al., 2008). In total there were five rootstock treatments: ‘Moneymaker’ non-grafted (‘Moneymaker’), ‘Moneymaker’ self-grafted (M/Money), ‘Moneymaker’ on ‘Multifort’ (M/Multifort), ‘Moneymaker’ on ‘Shield’ (M/Shield), and ‘Moneymaker’ on S. habrochaites (M/LA1777).

All seedlings were started in the optimal growth chamber. Seeds were sown 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 maintains similar physical properties as field soil and allows for thorough and clean extraction of roots (Manavalan et al., 2010; Surchoff et al., 2017). Following the recommendations of Venema et al. (2008) for successful grafting of LA 1777, seeds of the wild accession were sown 10 d prior to all other seeds due to its slow germination and initial growth. The non-grafted ‘Moneymaker’ control was sown 5 d after all rootstocks and self-grafted control to account for the 5 d healing process so that
all plants were at a similar physiological stage. Grafting utilizing the Japanese tube-graft method (Rivard and Louws, 2006) occurred when rootstock and scion hypocotyls were approximately 2 mm in diameter and had two- to three- true leaves. Grafts were healed in transparent plastic storage bins (67.3 cm L × 40.6 cm H × 31.8 cm W; Sterilite®, Townsend, MA) placed under a bench within the chamber where they received approximately 100 μmol·m⁻²·s⁻¹ PAR. During the 5 d healing process 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. All plants were transplanted 2 d after leaving the healing bins into 3.8 L plastic pots with dimensions of 20.3 cm (top diameter) x 20.3 cm (height) x 17.8 cm (bottom diameter) (8 Standard Growing Container; Belden Plastics, St. Paul, MN) lined with woven 20 × 20 mesh of 0.02 cm diameter thread (~0.016 cm² opening size; Clear Advantage Charcoal Fiber Glass Insect Screen, New York Wire, Hanover, PA) and filled with the calcined clay and sand media. The woven mesh kept the media from falling through the pot drainage holes and aids in root ball extraction.

**Experimental setup.** All plants were allowed to acclimate in the optimal chamber for 5 d after transplanting. Following this acclimation period, four replicates of each rootstock treatment were moved into the suboptimal and optimal chambers (n=20 pots per chamber). Pots were arranged in a completely randomized design within the chambers and moved daily to account for any potential light or air movement gradients. Plants were grown for 10 d in each chamber and fertilized twice (200 ppm of 20N-4.4P-16.6K; Peters Professional, JR Peters Inc., Allentown, PA) and watered as needed. Water and fertilizer temperatures were tempered to match that of the chamber in which they were used.
Data collection. Temperature sensors (MPS-6; METER Group, Pullman, WA) were placed in the center of two representative selected pots per chamber and soil temperature was collected using a data logger (EM50; METER Group). One day prior to termination of each trial leaf gas exchange and fluorescence measurements were collected. Measurements were taken on the terminal leaflet of the most recent fully expanded leaf using an open gas exchange system coupled with a leaf chamber fluorometer (LI-6800; Li-Cor, Inc., Lincoln, NE). Net CO₂ assimilation ($A$, $\mu$mol·m⁻²·s⁻¹), stomatal conductance ($g_s$, mmol·m⁻²·s⁻¹), effective quantum yield of photosystem II ($\phi_{PSII}$), and photochemical quenching ($q_P$) were measured midday on light-acclimated leaves. Photosynthetic photon flux density within the leaf chamber was set to 500 $\mu$mol·m⁻²·s⁻¹, with temperature and relative humidity maintained at levels matching those inside each growth chamber. The sensor head was left on each leaflet for two to three minutes until values of $A$ and $g_s$ stabilized. Minimal ($F_o$) and maximal ($F_m$) fluorescence values were obtained from dark-adapted leaves 3 h after lights turned off within the growth chambers. These values were used to calculated maximum quantum yield of photosystem II ($F_v/F_m$ where $F_v = F_m - F_o$). Upon termination of the study, the same leaf from which gas exchange and fluorescence data were collected was separated, it’s fresh weight and leaf area measured (LI-3100C area meter; Li-Cor, Inc.) then immediately frozen in liquid nitrogen. These samples were stored at -80 °C for analysis of sugar and starch concentrations. The remaining leaves from each plant were separated from the stem, weighed, and leaf area measured.

Starch and sugar quantification. Soluble sugars and starch content from freeze dried leaf tissue were determined utilizing high-performance liquid chromatography (HPLC) based on established protocols (Chow and Landhäusser, 2004; Smith and Zeetman, 2006; Warren et
al., 2015) with modifications. A 0.01 g sample of the ground tissue was mixed with 0.3 mL of 80% ethanol in a 2.0 mL centrifuge tube and incubated for 3 min. in a boiling water bath to stop enzymatic action. Following boiling, samples were allowed to cool to room temperature for approximately 5 min. Once cool, 0.7 mL of 80% ethanol was added to the sample and vortexed for 1 min., then centrifuged at 14,000 rpm at 4 °C for 20 min (5417R Refrigerated Centrifuge; Eppendorf, Hauppauge, NY). The resultant supernatant was collected. An additional 0.6 mL of 80% ethanol was added to the pellet which was vortexed for 1 min and centrifuged at 14,000 rpm at 4 °C for 20 min. The supernatant was collected and combined with the prior collected supernatant. Both the pellet and supernatant were dried completely for 80 min. in a vacuum concentrator (Savant™ DNA120 SpeedVac™ concentrator; Thermo Fisher Scientific, Waltham, MA).

The dried supernatant was vortexed for 1 min with 1 ml of distilled deionized water and filtered into HPLC vials with 0.2 µm filters (Target2™ Nylon Syringe filters; Thermo Fisher Scientific, Waltham, MA). A 5 µL aliquot was injected onto a Rezex RCM-Monosaccharide Ca+2 (8%), 00H-0130-KO (300x7.8 mm) column equipped with a Carbo-CA guard cartridge (Phenomenex, Torrance, CA) attached to a L 2130 pump (Hitachi High Technologies, San Jose, CA). The column was eluted with water at a flow rate of 0.6 ml/min and held at a temperature of 55°C. The soluble sugars glucose, fructose, and sucrose were detected using an IR detector (L-2490, Hitachi) at 45°C and quantified using standard curves from sucrose, glucose, and fructose (Sigma, St. Louis, MO). Chromatographic data were stored and processed (LaChrom Elite equipped with D-2000 software; Hitachi High Technologies, San Jose, CA).
Starch content was determined using the dried pellet. The material was resuspended in 0.5 mL H2O and sonicated for 10 min. (Branson 3510 Ultrasonic Cleaner; Branson, Danbury, CT). Starch within the sample was gelatinized by heating to 100 °C for 10 min. Samples were cooled for 5 min. and 0.5 mL of 200 mM sodium acetate (pH 5.5) added, followed by 200 μL (2 mg/ml) of amyloglucosidase (*Aspergillus niger*-derived; Sigma, St. Louis, MO) and 10 μL of α-amylase (100U porcine pancreas-derived; Millipore Sigma, St. Louis, MO). Tomato starch control tubes were prepared the same as the samples except contained solely 210 μL of 200 mM sodium acetate (pH 5.5). All tubes were incubated at 37 °C for 4 h, followed by centrifugation at 14,000 rpm for 20 min at 4 °C. Supernatants were filtered and run in the HPLC utilizing the above method for soluble sugars, and starch content was determined based on the glucose equivalents.

*Root system analysis.* Root balls were carefully excavated from the pot, rinsed free of media, and then placed in 0.5 g·L⁻¹ neutral red dye solution (Sigma Aldrich Co., St. Louis, MO) for 24 h at 6.7 °C. Following the dying process, roots were rinsed and placed in a 30 x 42 cm acrylic tray filled with 3 cm of water and scanned at 800 dots per inch using a flatbed scanner (Epson Expression® 10000XL, Epson America, Long Beach, CA). A root system image analysis software (WinRHIZO v. 2012b; Regent Instruments Inc. Quebec, Canada) was used to analyze the scanned images to obtain root morphological data including average root diameter, total root length, and length per diameter class (diameter classes were in increments of 0.5 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 diameter class length; RDCL). Roots were dried at 70 °C for 24 h and total root system dry weight used to calculate specific root length (total root length/root dry weight).
The study was repeated but temperatures were switched between chambers so that temperature was not nested within chamber.

Data analysis. Data were analyzed using the GLIMMIX procedure in SAS v 9.4 (SAS Institute Inc. Cary, NC). To avoid issues of pseudoreplication in the analysis, results were analyzed as a split-plot where temperature represented the whole plot and graft the split. The whole-plot error term was trial and the split-plot error term was chamber × trial. Proportion data (ϕPSII, qP, Fv/Fm, RDCL) were analyzed using a beta distribution and the Pearson chi-squared statistics divided by the degrees of freedom (φ) were checked for over-dispersion and distribution goodness-of-fit. Leaf area, total root length, and specific root length data showed strong heteroscedasticity which was ameliorated through square root transformations. All transformed data were back-transformed for presentation. Any effect found to be significant (P < 0.05) was further analyzed with Tukey’s honest significant difference post hoc mean separation test.

Results

Soils showed diurnal temperature variation for both trials (Fig. 1C and D). Though ambient temperatures in the suboptimal temperature chamber were maintained at 15 °C (Fig. 1A and B), soils showed warming due to absorption of light energy; however, the range and magnitude of this warming was lower than that of optimal temperature soils.

Shoot morphology and physiology. The interaction of graft and temperature was significant for total leaf area (Table 1). At optimal temperature there were no differences among ‘Moneymaker’, M/Money, or M/Multifort, all of which had significantly higher total leaf area compared to M/LA1777 (Fig. 2A). At suboptimal temperature there was a decline in total leaf area among all graft treatments except for M/Multifort, which maintained total leaf
area similar to all graft treatments at optimal temperature. Furthermore, leaf area of M/Multifort at suboptimal temperature was not significantly different from the same graft treatment at optimal temperature. Only the main effect of temperature affected stomatal conductance (Table 1); values at optimal temperature were higher than suboptimal (0.5604 mol·m⁻²·s⁻¹ and 0.3682 mol·m⁻²·s⁻¹, respectively; Table 2). Similarly, $qP$, $F_v/F_m$, and $\phi_{PSII}$ showed a significant depression at suboptimal temperature (Table 2). These photosynthetic responses were affected by graft treatments; values for all three were highest in M/Multifort. Values of $\phi_{PSII}$ and $qP$ for M/Multifort were significantly higher than ‘Moneymaker’ and M/Money (Table 3). Fewer differences were observed in $F_v/F_m$; ‘Moneymaker’ had a lower $F_v/F_m$ than M/Multifort (0.8337 and 0.8792, respectively; Table 2). $A$ was the only photosynthetic response affected by the interaction of graft and rootstock (Table 1). Values of $A$ were highest in M/Multifort at optimal temperatures compared to ‘Moneymaker’, M/Money, and M/LA1777 (Fig 2C). At suboptimal temperature these values dropped in all graft treatments. M/Multifort maintained the highest values of $A$ at suboptimal temperature compared to ‘Moneymaker’, M/Money, and M/Shield, though it was no different from M/LA1777.

The interaction of rootstock and temperature was significant for foliar starch concentration (Table 1). At optimal temperature there were no differences observed among the rootstock treatments; however, at suboptimal temperature M/LA1777 and M/Multifort had significantly lower starch concentrations compared to the remaining three rootstock treatments (Fig. 2B). There was no significant main effect or interaction for the individual or combined leaf soluble sugar concentrations (Table 1).
Root morphology. Average root diameter, total root length (TRL), and specific root length (SRL) were affected by the interaction of graft and temperature (Table 3). At optimal temperature TRL was similar for all graft treatments except for M/LA1777, which had a TRL significantly shorter than ‘Moneymaker’, M/Money, and M/Multifort (Fig. 4A). All graft treatments showed a significant drop in TRL at suboptimal temperature; however, M/Multifort maintained longer TRL than all other graft treatments. Furthermore, TRL values for M/Multifort at suboptimal temperature were no different than TRL for M/Shield and M/LA1777 at optimal temperature. Average root diameter was similar among ‘Moneymaker’, M/Money, and M/Shield at optimal temperature (Fig. 4B). At this temperature M/LA1777 and M/Multifort average root diameter values were similar and significantly thinner than the aforementioned three graft treatments. At suboptimal temperature all rootstock treatments showed a reduction in average root diameter with no differences observed among them. Additionally, the average root diameter of M/LA1777 and M/Multifort at optimal temperature were no different than the values of ‘Moneymaker’, M/Money, and M/Shield at suboptimal temperature. Values of SRL at optimal temperature were similar among all rootstock treatments (Fig. 4C). At suboptimal temperature, SRL dropped significantly in ‘Moneymaker’ and M/Money. M/Shield showed a similar reduction in SRL with suboptimal temperature however this drop was not statistically significant. Both M/LA1777 and M/Multifort showed no reduction in SRL with suboptimal temperature and maintained SRL similar to all rootstock treatments at optimal temperature.

Diameter class length 1 (DCL1) was affected by the rootstock × temperature interaction (Table 3). At optimal temperature all rootstock treatments had similar DCL1 values except for M/LA1777 (5082.75 cm; Table 4), which was significantly less than
‘Moneymaker’, M/Money, and M/Multifort. These values dropped with suboptimal temperature and at this temperature treatment M/Multifort maintained DCL1 values (5166.28 cm) significantly longer than all other rootstock treatments. This difference amounts to 42% to 56% more fine root length in M/Multifort compared to all other rootstock treatments at suboptimal temperatures. Relative diameter class length 1 (RDCL1) was similar among the rootstock treatments at optimal temperature and all showed a significant drop in RDCL1 at suboptimal temperature except for M/Multifort. This rootstock treatment was able to maintain similar RDCL1 values at optimal and suboptimal temperatures (0.7959 and 0.7945, respectively). DCL2 was affected by both the main effects of rootstock and temperature, but not the interaction (Table 3). DCL2 was highest at optimal temperature (1378.17 cm; Table 4). M/Multifort had the largest DCL2 (1412.13 cm), which was similar to ‘Moneymaker’ (1273.72 cm). The lowest DCL2 value was observed in M/LA1777 (997.86 cm). Unlike DCL2, RDCL2 was significantly affected by the rootstock × temperature interaction (Table 2). At optimal temperature M/LA1777 RDCL2 (0.1659; Table 4) was significantly higher than M/Money (0.1385). All rootstock treatments showed a significant increase in RDCL2 at suboptimal temperature and these values were similar among the rootstock treatments. However, RDCL2 for M/Multifort at suboptimal temperature (0.1816) was no different from all rootstock treatments at optimal temperature except for M/Money. DCL3 was affected by the main effects of rootstock and temperature and RDCL3 was only affected by temperature main effect (Table 3). DCL3 was highest at optimal temperature compared to suboptimal temperature (314.94 cm and 263.56, respectively; Table 4). Values of RDCL3 showed an opposite response, with suboptimal (0.0511) being higher than optimal (0.0350). Finally,
M/Multifort and ‘Moneymaker’ had the highest DCL3 values (356.66 cm and 318.81 cm, respectively). The lowest DCL3 was observed in M/LA1777 (221.53 cm).

Discussion

Results from this study indicate that the commercially available tomato rootstock ‘Multifort’ can reduce the amount of suboptimal temperature-induced shoot growth retardation (Fig. 2A and 3). In contrast with prior work (Venema et al., 2008; Ntatsi et al., 2014, 2017), no growth benefit was observed when utilizing LA 1777 as a rootstock in suboptimal temperatures. A general reduction in leaf area with M/LA1777 compared to the other rootstock treatments occurred; however, it should be noted that temperature-induced leaf area changes when using this rootstock were not significant (Fig. 2A). We attribute this general poor growth to graft incompatibility. Graft healing survival rate in M/LA1777 was low (20-30%; data not shown) compared to the other rootstock treatments (>90%). Bloom et al. (2004) had equal difficulty in successfully grafting onto LA 1777 and were unable to generate plants with this rootstock. In our trials, those plants that did survive showed unequal growth above and below the graft union (Fig. 5), which is indicative of graft incompatibility and poor development of vascular connections (Goldschimdt, 2014; Kawaguchi et al., 2008). As such, we reserve any conclusions on the suboptimal temperature tolerance of LA 1777 due to confounding issues of graft incompatibility.

Greenhouse production of tomatoes requires substantial fossil fuel-derived energy inputs. Increasing energy prices coupled with societal concerns regarding CO₂ emissions and its role in climate change require an improvement in energy use efficiency (Ntatsi et al., 2014). Relatively small drops in greenhouse temperature settings can result in significant energy savings. Reducing daytime high temperatures by 2 °C (19 °C to 17 °C) resulted in a
16% savings in energy costs though it did reduce yearly production by 3.3% (Elings et al., 2005). Breeding of more productive tomato varieties has improved energy-use efficiency two-fold; however, this improvement is due not to an increase in suboptimal temperature tolerance but an overall increase in yield per unit energy (van der Kniff et al., 2004; Venema et al., 2008).

Breeding efforts directed at improving suboptimal temperature tolerance in *S. lycopersicum* roots and shoots is hindered by lack of genetic variability (Nieuwenhof et al., 1993, 1997, 1999). Wild tomato relatives show a wider range of optimal temperatures for growth and reproduction compared to *S. lycopersicum* (Venema et al., 2005). As discussed prior, when used as a rootstock, *S. habrochaëtes* LA 1777 improves suboptimal tolerance in susceptible scions. Compared to traditional cultivar breeding, grafting has the benefit of being customizable; the grower can select the scion variety to meet the market demand while selecting the rootstock to meet the disease pressure and abiotic stress.

LA 1777 root systems have been investigated regarding the mechanisms for improved cold tolerance. Venema et al. (2008) observed less suboptimal temperature-induced inhibition of root growth in LA 1777 rootstock compared to ‘Moneymaker’. Our results with ‘Multifort’ are in agreement with these observations; while all rootstock treatments showed a reduction in TRL with suboptimal temperature, M/Multifort maintained TRL significantly higher than all other treatments (Fig. 4A). More importantly, M/Multifort maintained a high proportion of its TRL as roots with very thin diameter (RDCL1) while all other rootstocks showed a reduction in RDCL1 (Table 4). At suboptimal temperature, M/Multifort had 5166.28 cm of roots with diameters less than 0.5 mm (DCL1; Table 4). All other rootstock treatments at suboptimal temperature had DCL1 values ranging from 3320.98 to 3638.27 cm.
This amounts to M/Multifort having 42% to 56% more fine root length compared to the other rootstock treatments. Root systems composed of thin diameter roots maintain higher hydraulic conductivity due to their reduced radial hydraulic resistance and improved absorption (Ho et al., 2005; Huang and Eissenstat, 2000; Rieger and Litvin, 1999). Suboptimal soil temperatures lead to increased viscosity of soil water and consequent reduction in root hydraulic conductance (Equiza et al., 2001). The ability for M/Multifort to produce higher amounts of very thin roots, with improved absorption and hydraulic conductance, may be one of the mechanisms that allows it to tolerate suboptimal temperatures and compensate for increased water viscosity. These results may also explain the improved leaf turgor observed in susceptible tomato lines grafted onto \textit{S. lycopersicum} × \textit{S. habrochaites} introgressions at suboptimal rootzone temperatures (Easlon et al., 2013).

While not investigated in this study, molecular and anatomical attributes of ‘Multifort’ root systems are needed. Grafting cucumber (\textit{Cucumis sativus}) scions onto figleaf gourd (\textit{Cucurbita ficifolia}) rootstocks can improve growth, photosynthesis, and yield at suboptimal soil temperatures (Ahn et al. 1999; Zhou et al., 2007). These improvements in scion cold tolerance have been attributed to specific traits in the morphology and physiology of the figleaf gourd root system such as increased unsaturated fat deposition in cell lipid membranes (Lee et al., 2005a) and reduction in cold-induced suberin deposition and increased aquaporin activity (Lee et al., 2005b). These responses allow fig-leaf gourd root systems to maintain high root hydraulic conductivity and consequent movement of nutrients at lower temperatures compared to susceptible cucumber roots (Ahn et al., 1999; Lee et al., 2005b). More work is warranted to determine if the lipid composition or aquaporin activity differs between \textit{S. lycopersicum} and \textit{S. habrochaites} or rootstocks such as ‘Multifort’.
containing S. habrochaites parentage. Furthermore, recent reports indicate the importance of small peptides as signaling molecules between shoot and root in root system development under situations of limited nitrogen or abiotic stress (Oh et al., 2018). Future work is needed in determining whether the quantities and types of signaling peptides differ among tomato rootstocks and how they change under conditions of abiotic stress and limited resources.

The photosynthetic apparatus is sensitive to suboptimal temperatures and changes in efficiency due to reduced temperatures can be easily observed through chlorophyll fluorescence (Allen and Ort, 2001; Kingston-Smith et al., 1997; Lynch, 1990; Maxwell and Johnson, 2000). \( F_v/F_m \) is a measure of intrinsic PSII efficiency and, because PSII efficiency reduces with suboptimal temperatures, values of \( F_v/F_m \) can be compared to determine the effect of temperature stress. We observed this temperature-dependent reduction in \( F_v/F_m \) (Table 2). Interestingly, there was a constitutive rootstock effect on \( F_v/F_m \); M/Multifort had higher \( F_v/F_m \) (0.8792) compared to ‘Moneymaker’ (0.8337). This constitutive rootstock effect on \( F_v/F_m \) was observed by Albacete et al. (2009) with rootstocks from recombinant inbred lines of S. lycopersicum × S. cheesmaniae. Those rootstocks which improved leaf fresh weight and area also had significantly higher \( F_v/F_m \) compared to non- and self-grafted controls regardless of salinity stress. Though a different genus, our results are in agreement with Ahn et al. (1999) who found that \( F_v/F_m \) in cucumber leaves was improved at suboptimal temperatures when grafted onto the cold tolerant figleaf gourd rootstock. Because intrinsic PSII efficiency was higher in M/Multifort, we also observed a higher proportion of quantum energy used for photochemistry (\( \Phi_{PSII} \)) and open PSII reaction centers (\( q_P \)): Table 2). Ntatsi et al. (2017) did not observe an effect of temperature or rootstock on photosynthetic efficiency, CO₂ assimilation rate, or leaf soluble carbohydrates. These researchers used a
hydroponic system to apply nutrient solutions of differing temperatures (15 °C or 25 °C) directly to the root zone; however, ambient temperatures were similar (~ 25 °C). Tomato photosynthetic efficiency is reduced in suboptimal ambient temperatures (Walker et al., 1990). Consequently, both ambient and root zone temperatures should be considered when comparing rootstock effects on photosynthesis and gas exchange.

Suboptimal temperature reduced stomatal conductance equally among the rootstock treatments (Table 1 and 2). Regardless, M/Multifort maintained higher CO2 assimilation rates at suboptimal temperature (Fig. 2C). Taken together, the longer root system in M/Multifort at suboptimal temperature (Fig. 4A) may have maintained carbohydrate sink strength in the roots, allowing for proper movement of photosynthate from leaves to roots, which can be observed in the reduced foliar starch concentration (Fig. 2B). Increased starch concentrations can induce feedback inhibition of photosynthesis (Goldschmidt and Huber, 1992; Paul and Foyer, 2001). The reduced starch concentrations in M/Multifort at suboptimal temperature (Fig. 2B) allowed for increased photosynthetic efficiency and net CO2 assimilation (Table 2 and Fig. 2C), and thus maintaining proper growth as seen in leaf area production (Fig. 2A).

The results of this study indicate ‘Multifort’ can improve tolerance to suboptimal temperatures at early stages of plant development. The ability to maintain growth for field-grown tomatoes when soil temperatures are suboptimal may allow growers to meet more lucrative, early-season markets. Incorporation of no-till practices in warm-season vegetable production is limited due to lower soil temperatures early in the season (Hoyt et al., 1994). Utilization of cold-tolerant rootstocks in no-till systems may offer growers the ability to overcome the suboptimal soil temperatures associated with residue mulches.
Literature Cited:


Table 1. Results of analysis of variance for the impact of rootstock and temperature on tomato shoot morphology and physiology.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Leaf Area</th>
<th>$g_s^z$</th>
<th>$A^y$</th>
<th>$\Phi_{\text{PSII}}^x$</th>
<th>$F_v/F_m^w$</th>
<th>$qP^w$</th>
<th>Sucrose$^u$</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Total soluble sugars</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td>4</td>
<td>*** NS</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>*** ***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Graft × Temperature</td>
<td>4</td>
<td>* NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at $P \leq 0.05$, significant at $P \leq 0.05$, 0.01, 0.001, respectively.

$^z$Stomatal conductance.

$^y$Net CO$_2$ assimilation rate.

$^x$Effective quantum yield of photosystem II

$^w$Maximum quantum yield of photosystem II

$^v$Photochemical quenching

$^u$Soluble sugars (sucrose, glucose, fructose) and starch concentrations measured from a single leaf via HPLC.
Table 2. Main effects of grafting and temperature on photosynthesis and gas exchange in tomatoes grown in a controlled environment.

<table>
<thead>
<tr>
<th>Graft</th>
<th>$g_s^z$ (mol·m$^{-2}·s^{-1}$)</th>
<th>$\Phi_{\text{PSII}}^y$</th>
<th>$F_v/F_m^x$</th>
<th>$qP^w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Moneymaker’</td>
<td>0.3906 b$^v$</td>
<td>0.8337 b</td>
<td>0.6525 b</td>
<td></td>
</tr>
<tr>
<td>‘Moneymaker’/ ‘Moneymaker’</td>
<td>0.3785 b</td>
<td>0.8527 ab</td>
<td>0.6552 b</td>
<td></td>
</tr>
<tr>
<td>‘Moneymaker’/ ‘Shield’</td>
<td>0.4182 ab</td>
<td>0.8530 ab</td>
<td>0.6997 ab</td>
<td></td>
</tr>
<tr>
<td>‘Moneymaker’ / LA1777</td>
<td>0.4087 ab</td>
<td>0.8488 ab</td>
<td>0.6933 ab</td>
<td></td>
</tr>
<tr>
<td>‘Moneymaker’/ ‘Multifort’</td>
<td>0.4709 a</td>
<td>0.8792 a</td>
<td>0.7423 a</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>0.5604 a</td>
<td>0.5348 a</td>
<td>0.8775 a</td>
<td>0.7839 a</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>0.3682 b</td>
<td>0.3043 b</td>
<td>0.8272 b</td>
<td>0.5764 b</td>
</tr>
</tbody>
</table>

$^z$Stomatal conductance.

$^y$Effective quantum yield of photosystem II

$^x$Maximum quantum yield of photosystem II

$^w$Photochemical quenching

$^v$Means followed by the same letter within response and main effect are not different (Tukeys HSD; $\alpha = 0.05$) and represent the average of two trials, four replicates, and two temperature treatments for the graft main effect (n=16 data points for each mean) and two trials, four replicates, and five graft treatments for the temperature main effect (n=40 data points for each mean).
Table 3. Results of analysis of variance for the impact of rootstock and temperature on tomato root system morphology.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Average Root Diameter</th>
<th>Total Root Length</th>
<th>Specific Root Length&lt;sup&gt;υ&lt;/sup&gt;</th>
<th>DCL1&lt;sup&gt;x&lt;/sup&gt;</th>
<th>DCL2</th>
<th>DCL3</th>
<th>RDCL1&lt;sup&gt;w&lt;/sup&gt;</th>
<th>RDCL2</th>
<th>RDCL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td>4</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>***</td>
<td>***</td>
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<td>***</td>
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<td>***</td>
</tr>
<tr>
<td>Graft × Temperature</td>
<td>4</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, *, **, *** denote non-significant at \( P \leq 0.05 \), significant at \( P \leq 0.05, 0.01, 0.001 \), respectively.

<sup>2</sup>Ratio of root dry weight to shoot dry weight.

<sup>υ</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.
Table 4. Effect of grafting and temperature on diameter class length and relative diameter class length proportions for tomatoes grown in a controlled environment.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Graft</th>
<th>DCL1*</th>
<th>DCL2</th>
<th>DCL3</th>
<th>RDCL1*</th>
<th>RDCL2</th>
<th>RDCL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>‘Moneymaker’</td>
<td>7781.74 a</td>
<td>0.8010 a</td>
<td>0.1562 bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. Mooney</td>
<td>7914.19 a</td>
<td>0.8302 a</td>
<td>0.1385 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. Shield</td>
<td>6456.52 ab</td>
<td>0.8063 a</td>
<td>0.1633 bc</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>M. L. A. 777</td>
<td>5622.79 bc</td>
<td>0.8069 a</td>
<td>0.1690 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-optimal</td>
<td>‘Moneymaker’</td>
<td>7045.35 a</td>
<td>0.7959 ab</td>
<td>0.1635 bc</td>
<td></td>
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<tr>
<td></td>
<td>M. Mooney</td>
<td>3638.27 ed</td>
<td>0.7437 c</td>
<td>0.2024 a</td>
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<tr>
<td></td>
<td>M. Shield</td>
<td>3474.33 ed</td>
<td>0.7440 c</td>
<td>0.2046 a</td>
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<tr>
<td></td>
<td>M. L. A. 777</td>
<td>3320.98 d</td>
<td>0.7495 bc</td>
<td>0.2049 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. McHaff</td>
<td>5166.28 b</td>
<td>0.7385 ab</td>
<td>0.1816 ab</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Main effects

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Graft</th>
<th>DCL1</th>
<th>DCL2</th>
<th>DCL3</th>
<th>RDCL1</th>
<th>RDCL2</th>
<th>RDCL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>‘Moneymaker’</td>
<td>1378.17 A</td>
<td>314.94 A</td>
<td>998.94 B</td>
<td>263.56 B</td>
<td>0.0356 B</td>
<td>0.0511 A</td>
</tr>
<tr>
<td>Sub-optimal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter within DCL or RDCL are not different (Tukeys HSD; α = 0.05) and represent the average of two trials, four replicates and two temperature treatments for graft main effect (n=16 data points for each mean) and two trials, four replicates, and five graft treatments for the temperature main effect (n=40 data points for each mean), and two trials and four replicates (n=8 data points for each mean) for the interaction of graft and temperature.

*Diameter class length. DCL1 is the length of roots with diameter less than 0.5 mm, DCL2 is the length of roots with diameter between 0.5 and 1.0 mm, and DCL3 are roots with diameter greater than 1.0 mm.

*Relative diameter class length. RDCL1 is the proportion of total root length composed of roots with diameters less than 0.5 mm, RDCL2 is the proportion of total root length composed of roots with diameter between 0.5 and 1.0 mm, and RDCL3 are roots with diameter greater than 1.0 mm.
Figure 1. Trial 1 ambient (A) and soil (C) temperatures and trial 2 ambient (B) and soil (D) temperatures. Soil temperatures were measured with matric water potential and temperature sensors (MPS-6; METER Group, Pullman, WA) placed in the center of two pots per chamber per trial and stored in a data logger (EM50; METER Group, Pullman, WA).
Figure 2. Effect of grafting and temperature on total leaf area (A), foliar starch concentration (B) and net CO₂ assimilation rate (C) ± standard error. Means with common letters within a response are not different (Tukeys HSD; α = 0.05) and represent the average of four replicates and two trials (n=8 data points for each mean). Rootstock treatments include non-grafted ‘Moneymaker’, self-grafted ‘Moneymaker’ (M/Money), ‘Moneymaker’ grafted onto *S. habrochaites* LA 1777 (M/LA1777), ‘Moneymaker’ grafted onto ‘Shield’ rootstock (M/Shield), and ‘Moneymaker’ grafted onto ‘Multifort’ rootstock (M/Multifort). Temperature regimes were 25 °C day / 20 °C night (optimal) and 15 °C day / 15 °C night (suboptimal).
Figure 3. Grafted tomatoes grown at optimal temperature (25 °C Day / 20 °C Night; A) and suboptimal temperature (15 °C Day / 15 °C Night; B). Rootstock treatments include non-grafted ‘Moneymaker’ (‘Moneymaker’), self-grafted ‘Moneymaker’ (M/Money), ‘Moneymaker’ on ‘Shield’ (M/Shield), ‘Moneymaker’ on S. habrochaites accession LA 1777 (M/LA1777), and ‘Moneymaker’ on ‘Multifort’ (M/Multifort).
Figure 4. Effect of grafting and temperature on total root length (A), average root diameter (B), and specific root length (C) ± standard error. Means with common letters within a response are not different (Tukeys HSD; α = 0.05) and represent the average of four replicates and two trials (n=8 data points for each mean). Rootstock treatments include non-grafted ‘Moneymaker’, self-grafted ‘Moneymaker’ (M/Money), ‘Moneymaker’ grafted onto S. habrochaites LA 1777 (M/LA1777), ‘Moneymaker’ grafted onto ‘Shield’ rootstock (M/Shield), and ‘Moneymaker’ grafted onto ‘Multifort’ rootstock (M/Multifort).

Temperature regimes were 25 ºC day / 20 ºC night (optimal) and 15 ºC day / 15 ºC night (suboptimal).
Figure 5. Graft union of ‘Moneymaker’ scion on *Solanum habrochaites* accession LA 1777 rootstock showing signs of graft incompatibility.