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Evaluating the Impact of Grafting on Local Tomato Production in

Nebraska

By

Raihanah Hassim

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Evaluating the Impact of Grafting on Local Tomato Production in

Nebraska

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University of Nebraska, 2020

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Vegetable grafting has been known to improve plant production under both biotic and abiotic stresses. With an increase in interest among local growers towards grafting production, it is important to provide enough vegetable grafting information. Therefore, the objective of this study is to assess the impact of grafting, rootstock cultivar, and local conditions and management on the yield and quality of tomato across the diverse growing and environmental conditions, specifically in Nebraska. Three open-field and one limited growing condition study were conducted between 2018 and 2019 across Nebraska. In the open-field trial, two determinant fresh market tomatoes, 'Nebraska Wedding' and 'BHN-589', were grafted onto one of two rootstocks, 'Estamino' and 'Maxifort,' with the nongrafted scion cultivars as controls. In 2019, a fertilizer treatment was introduced at all three locations with two different Nitrogen (N) rates (0 and 50 kg N ha⁻¹). In the limited growing condition trial that took place in a greenhouse at the University of Nebraska, Lincoln, 'BHN-589' were grafted onto 'Estamino' and 'Maxifort,' with the nongrafted scion cultivars as controls. Nitrogen (N) fertilizer treatments were implemented at 0.5 X, 1.0 X, and 1.5 X of 120 ppm of N, and water treatment was divided into high (above field capacity) and low (below field capacity). Overall, grafting did not

provide consistent yield benefits under both trials. Under the open field condition, in 2018, nongrafted 'BHN-589' increased the number of marketable fruits by 54%. Whereas, in 2019, 'BHN-589' grafted onto 'Maxifort' increased total yield by 24%. Under the limited growing condition trial, 'Estamino' improved % of fruits marketability by 28% compared to the nongrafted plants, especially under 1.5 X of N fertility treatment. However, there were no significant differences in total and marketable yield between grafted and nongrafted plants. Moreover, there was no interaction effect between grafting and fertilizer treatment under both trials. Results from this study suggest the need for more assessment on the impact of field tomato grafting under different environmental conditions.

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Dedication

I dedicate this work to my beloved family and friends. Abah and mama, this is for you; thank you so much for the do'a and love. To my siblings, I hope I set an excellent example and that you guys are proud of me. To Jason, thank you for being there with me throughout this rough time; I wouldn't be where I am at today if it wasn't for your support.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Chapter 1 – The Impact of Grafting on Hybrid and Heirloom

Tomato Yield in Local Open Field Production

Abstract

Grafting has been successfully used in vegetable production for tomatoes, peppers, eggplants, cucumbers, and watermelon. Besides its usefulness for managing biotic and abiotic stresses, grafting is well known for its capability to improve yield and nutrient uptake. However, few studies have assessed the effects of grafting in an open field-grown tomatoes in the Midwest, especially in Nebraska. Therefore, the objective of this two-year research project was to better document the effects of grafting heirloom and hybrid tomato cultivars onto interspecific hybrid tomato rootstocks by measuring tomato yield and quality. The field experiments were located at the University of Nebraska Lincoln - East Campus in Lincoln, West Central Research and Extension Center in North Platte, and at Perkarek's Produce vegetable farm near Dwight, Nebraska. Two determinant fresh market tomatoes, 'Nebraska Wedding' and 'BHN-589', were grafted onto one of two potentially valuable rootstocks, 'Estamino' and 'Maxifort,' with the nongrafted scion cultivars as controls. During the second year of the study, a fertilizer treatment was introduced with two N rates (0 and 50 kg N ha⁻¹). At the end of the growing season, ripe tomatoes were harvested weekly, and yield was determined by weighing all tomatoes from the five plants in each experimental unit. Overall, there was no consistent improvement in yield for any of the grafting treatments. In 2018, nongrafted 'BHN-589' increased the number of marketable fruits by 54%. Whereas, in 2019, 'BHN-589' grafted onto 'Maxifort' increased total yield by 24%. Moreover, there was no interaction effect between grafting and fertilizer treatment within each location. Results from this study suggest the need for more assessment on the impact of field tomato grafting under different environmental conditions.

Introduction

Tomato (*Solanum lycopersicum*) is considered one of the world's main specialty crops, with global production surpassing 182 million metric tons in 2017 (FAOSTAT, 2020). The U.S. produces more than 12.6 million tons of tomatoes each year and is, therefore, one of the world's leading producers of this specialty crop (FAOSTAT, 2020). Tomato can be grown in both field and protected production environments such as high tunnel. Numerous studies have documented the benefits of growing tomatoes in a high tunnel production system (Galinato and Miles, 2013; Lang, 2019). One of the primary benefits is the possibility of earlier planting dates due to an extended growing season (O'Connell et al., 2012; Wells and Loy, 1993), and high tunnel production allows farmers to mitigate the impacts of extreme weather on their high value crops (Both et al., 2007; Carey et al., 2009; Djidonou et al., 2020; Lamont, 2009; Meyer, 2016; Schwarz et al., 2013). However, high tunnel production requires a significant investment amount, including the initial cost for the high tunnel structure and ongoing maintenance (Meyer, 2016). Plus, farmers are confined to one specific growing space within the tunnel, which results in increased soilborne disease in that area (Meyer, 2016).

Despite the increasing popularity of high tunnel systems, most vegetables produced in the U.S. are still grown under open-field conditions (FAOSTAT, 2020) due in large part to inadequate technical information and the high capital cost (Meyer, 2016; Lang, 2019). Though less expensive, open-field production leaves crops vulnerable to exposure to biotic and abiotic stresses, such as unpredictable weather events, drought, soil salinity, extreme temperature, and pathogens. Schwarz et al. (2013) suggested that stress-resistant crops were needed for field production systems, but the process of breeding a new plant can be

lengthy and costly (Hayward et al., 2012; Schwarz et al., 2013; Venema et al., 2011). An alternative solution to managing abiotic and biotic stress in field production systems is grafting.

Grafting is a propagation method that combines a desirable shoot cultivar (scion) with a root system (rootstock) that results in a hybrid plant with desirable agronomic or horticultural traits. Both the scion and the rootstock are selected based on a unique trait of interest. For example, the scion may be selected based on distinct fruit yield and unique flavor profile. In contrast, the rootstock may be selected based on having higher root density and resistance to pathogens. The propagation method of grafting has been practiced on fruit trees since 2500 BC in Mesopotamia, and the method then made its way through the Roman Empire and survived the Dark Ages (Hartman et al., 2002; Masterson et al., 2016; Mudge et al., 2009; Virgil, 1953). Grafting vegetable crops has recently gained farmer's interest as it helps produce plants that combine the beneficial traits of two plants into one in a matter of a few days compared to plant breeding, which will take years (Flores et al., 2010; Hu, 2016; Masterson et al., 2016; Meyer, 2016).

Vegetable grafting was first practiced in an attempt to improve crop resistance against soilborne diseases in Japan and Korea (Lang, 2019; Lee et al., 2010). Grafting a desired cultivar with a resistant rootstock can be very effective and eco-friendly when it comes to suppressing soilborne diseases. These diseases include root-knot nematodes (*Meloidogyne spp.*), bacterial wilt (*Ralstonia solanacearum*), verticillium wilt (*Verticillium dahliae*), fusarium wilt (*Fusarium oxysporum*), southern blight (*Sclerotium rolfsii*), Tomato Mosaic Virus, and Tomato Spotted Wilt Virus (Lee et al., 2010). Multiple studies have observed a lower disease incidence when grafting scion cultivars with resistant rootstocks (Barrett et

al., 2012; Mc Avoy et al., 2012; Rivard and Louws, 2008). Under a few circumstances, grafting has been shown to save farmers hundreds of dollars per acre when dealing with a severely infested field (Barrett et al., 2012; Rysin et al., 2015). Rysin et al., 2015 had observed a positive net revenues in the grafted system ranging from \$108 to \$12,328 per acre given grafted plants produce higher marketable fruits compared to nongrafted plants. Most of these studies also noted that the severity of pest infestation in a field plays an important role in realizing significant yield improvements from grafting (Barrett et al., 2012; Mc Avoy et al., 2012; Rivard and Louws, 2008; Rysin et al., 2015).

For various ornamental and specialty crops, grafting is an essential tool that can be used to combat abiotic stresses (Flores et al., 2010; Savvas et al., 2010). Specifically under field production, tomato plants are exposed to various abiotic environmental challenges, including extreme soil temperature, drought, and nutrient depletion. Grafting a specific desirable cultivar to rootstocks with greater root mass and root length may result in improved nutrient uptake (Djidonou et al., 2013) and tolerance for hot and dry environments (Abdelmageed et al., 2009; Sánchez-Rodríguez et al., 2011). With this knowledge in mind, organic farmers specializing in heirloom tomato production may benefit from grafting.

‘Nebraska Wedding’ is an old Great Plains tomato heirloom that was brought to western Nebraska when the Homestead Acts were enacted in 1862 (Dwivedi et al., 2019). The name of the heirloom was given based on a Nebraska tradition where a newlywed was given ‘Nebraska Wedding’ tomato seeds as a wedding gift. Heirloom tomatoes, such as ‘Nebraska Wedding,’ are often more susceptible to disease and abiotic stress (Masterson et al. 2016; Bai et al. 2007; Rivard and Louws, 2008). Heirloom cultivars do not have the

disease and pest resistance traits that would typically be accumulated over generations of selective breeding, cross-pollination, or hybridization (O'Connell et al. 2012; Rivard and Louws, 2008). Therefore, grafting can be a very useful tool for local growers to improve agronomic performance of heirloom tomato plants while maintaining the fruit's unique flavor, color, and texture (Rivard and Louws, 2008; Rivard et al., 2010; Barrett et al., 2012). 'BHN-589' is one of the most popular tomato hybrids produced commercially in Nebraska, and Loewen (2018) observed a "highly compatible" interaction between 'BHN-589' and 'Maxifort.'

'Maxifort' is widely used in the grafting industry and is claimed as the "standard" tomato rootstock in a meta-analysis that was carried out in California (Grienesen et al., 2018). 'Maxifort' improves plant performance by providing the scion with a vigorous and disease-resistant rootstock (Buller et al., 2013; Hu, 2016; Loewen, 2018; Masterson et al., 2016; Meyer, 2016). Although 'Maxifort' is well known for its ability to suppress soilborne diseases (Rivard and Louws, 2008; Rivard et al., 2010), few studies have reported yield improvements from grafted plants in fields with little to no disease pressure (Lang, 2019; Meyer, 2016). 'Estamino' is a relatively new and improved rootstock in the market (Lang, 2019). Rivard et al. (2011) had reported 'Estamino' to be resistant towards diseases such as root-knot nematodes, verticillium wilt, fusarium wilt race 1, 2, and 3, Tomato Mosaic Virus, and Tomato Spotted Wilt Virus. It is unknown whether the 'Estamino' rootstock can improve tomato productivity in field environments, particularly in the absence of common soil-borne pathogens.

Several recent studies and surveys have reported an increase in interest among local farmers and small growers in grafted vegetable production, specifically in the Midwest

(Hu, 2016; Meyer, 2016). The knowledge of grafting tomatoes is well understood in major vegetable producing states such as California and Florida. However, there is a research gap of grafting tomatoes adapted to the farming system in Nebraska. Local growers have limited knowledge about whether grafting can improve their tomato production and what rootstock selections may be most compatible with the local hybrid ‘BHN-589’ and the local heirloom ‘Nebraska Wedding.’ While many studies have assessed grafted tomato performance in the Midwest, most were conducted in a protected growing system such as a high tunnel (Lang, 2019; Loewen, 2018; Masterson et al., 2016; Meyer, 2016). Therefore, this research aims to assess the impact of grafting, rootstock cultivar, and local conditions and management on the yield and quality of tomato across diverse field environments in Nebraska. This research also hypothesized that grafting will provide tomato plants with improved yield and nutrient uptake under different field environment.

Materials and Methods

Experimental Design

In 2018, a 2×3 factorial treatment structure was used in a randomized complete block design across three locations (Lincoln, North Platte, and Perkarek’s Produce, NE). The treatment factors were scion cultivar (‘Nebraska Wedding’ and BHN 589’), rootstock cultivar (‘Maxifort,’ ‘Estamino,’ and a nongrafted control), and location (Lincoln, North Platte, and Perkarek’s Produce, NE). In 2019, a $2 \times 3 \times 2$ factorial treatment structure was used in a randomized complete block design across the same three locations. The treatment factors were scion cultivar (‘Nebraska Wedding’ and BHN 589’), rootstock cultivar (‘Maxifort,’ ‘Estamino,’ and a nongrafted control), and fertilizer (0 or 50 kg ha⁻¹ N).

Site Descriptions

Field experimental sites were located at the University of Nebraska Lincoln - East Campus, in Lincoln, Nebraska (Lat. 40.82 ° N, Long. 96.70 °W), the University of Nebraska West Central Research and Extension Center in North Platte, Nebraska (Lat. 41.14° N, Long. 100.76° W), and at Perkarek's Produce (a local diversified vegetable farm) near Dwight, Nebraska (Lat. 41.08° N, Long. 97.02° W). The dominant soil at the University of Nebraska Lincoln - East Campus (LNK) is a Zook silty clay loam (Fine, smectitic, mesic Cumulic Vertic Endoaquolls) with 0 to 2% slope; soil at the West Central Research and Extension Center in North Platte (NP) is a Cozad silt loam (Coarse-silty, mixed, superactive, mesic Typic Haplustolls) with 0 to 1% slope; and soil at Pekarek's Produce in Dwight (PP) is a Hastings silt loam (Fine, smectitic, mesic Udic Argiustolls) with 1 to 3% slope. Locations were chosen to include a diversity of climate and soil characteristics in the experiment (Figure 1.1, Table 1.1).

Grafting Treatment

Scion cultivars included the local heirloom tomato, 'Nebraska Wedding,' and a local grower-favorite commercial hybrid, 'BHN-589'. Scions from these cultivars were grafted onto 'Maxifort' (BHN-MAX; NW-MAX), a popular vegetative rootstock hybrid, and 'Estamino' (BHN-EST; NW-EST), an organic, generative rootstock hybrid that puts greater energy into fruit production (Johnny's Selected Seeds, Winslow, ME). Nongrafted 'BHN-589' (BHN-NON) and nongrafted 'Nebraska Wedding' (NW-NON) were included as controls that helped in evaluating differences in yield between grafted and nongrafted tomatoes. Previous experiments with Nebraska Wedding suggested there is no yield

penalty associated with grafting itself, as determined from a self-grafted control (unpublished data); thus, no self-grafted controls were included in these experiments.

Fertility Treatment

In 2019, tomatoes received one of two in-season fertilizer treatments, including zero and full nitrogen via fertigation. The full nitrogen application rate was determined based on a recommendation of 50 kg ha⁻¹ N for field tomato production and was applied via hand fertigation as calcium nitrate [Ca(NO₃)₂] (15.5N-0P-0K+26.5 CaO; Hummert International, Topeka, KS). To prepare the fertigation solution, 1.7 kg calcium nitrate was mixed with 120 liters of water. One liter of the fertigation solution was applied by hand adjacent to individual plants beneath the plastic mulch film. Fertilizer treatment began one week after seedlings were transplanted: 7 June for LNK, 13 June for PP, and 18 June for NP. Both NP and PP received a total of five fertigation treatments and ended on 31 July and 8 Aug. when the plants started to fruit. Lincoln received a total of six fertigation treatments that ended on 8 August.

Grafting Procedure

The germination and emergence of rootstock seeds took longer than the scion seeds; therefore, rootstock seeds were sown on 5 Mar. 2018 and 26 Mar. 2019 – three days earlier than the scion seeds, which were sown on 8 Mar. 2018 and 29 Mar. 2019. This was done to increase the likelihood that the diameter of scion and rootstock seedling stems would be similar, to increase the grafting success rate. The seedlings were grown in an environmentally controlled greenhouse with the temperature set between 26.7 to 32.2 °C

during the daytime and 15.6 to 21.1 °C during the night. HID sodium halide lamps were provided in this greenhouse that would activate at night and on cloudy days. Seedlings were sown in 72-cell plug trays with the cells measured at 38 mm × 38 mm × 57 mm deep, filled with a soilless potting mixture including coarse grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone, non-ionic wetting agent and standard fertilizer starter charge (BM1; JR Johnson, St. Paul, Minnesota). Approximately three weeks after seeding, on 10 Apr. 2018 and 23 Apr. 2019, scions from both ‘Nebraska Wedding’ and ‘BHN-589’ were grafted onto the rootstocks ‘Maxifort’ and ‘Estamino.’ The grafting work area was disinfested with isopropyl alcohol before and during the grafting session.

To begin the grafting procedure, shoots of the rootstocks were first removed below the cotyledons at a 45° angle using a miter-cut grafting knife (Johnny’s Selected Seeds, Winslow, ME). Next, an identical 45° angle cut was made of the scions using the same knife. The scion and rootstock stems were carefully joined together and secured with a 1.5 mm diameter silicon tube (Johnny’s Selected Seeds, Winslow, ME). After grafting, plants were immediately transferred into a closed healing chamber built of polyvinyl chloride pipe and clear polyethylene plastic. Chambers were equipped with a thermometer, two humidifiers, and a relative humidity sensor. Light transmission was filtered from the chamber using white linens for the first seven days after the grafted tomatoes were transferred into the chamber. Additional layers of linen were removed daily after seven days to allow the grafted tomatoes to acclimate to ambient greenhouse conditions. Temperature and average relative humidity of the healing chamber were maintained between 21 to 27 °C and >90% humidity in the first three days. On 13 Apr. 2018 and 26

Apr. 2019, the humidity level was reduced to 70%. Ten days later, relative humidity was reduced by opening up the chamber and increasing light exposure. Grafted plants were watered gently at the base of the plants as needed, and adventitious roots were removed as needed. Two weeks after grafting, plants were moved from the healing chamber and into the greenhouse alongside the nongrafted seedlings. After that, all plants were fertigated with a 20N-10P-20K fertilizer solution every week until transplanting in the field.

Crop Management

In both years, field plots were cultivated with a disc implement on all three locations before transplanting took place on 21 May 2018 and 31 May 2019 at LNK, 24 May 2018 and 11 June 2019 at NP, and 22 May 2018 and 5 June 2019 at PP. Soil samples (0-15 cm depth) were collected annually at each location and analyzed to determine fertilizer application needs. Based on these analyses, nitrogen (applied as $\text{Ca}(\text{NO}_3)_2$), inorganic phosphorus (P_2O_5), and inorganic potassium (K_2O) fertilizers were applied preplant as needed based on the soil test results. Granular inorganic phosphorus (P_2O_5) was broadcast-applied preplant at PP in 2018 and 2019. At NP, P_2O_5 and K_2O were applied in both years – via fertigation in the crop planting hole in 2018 and as a granular pre-plant in 2019. At LNK, P_2O_5 and K_2O were applied in 2018 via fertigation in the crop planting hole. In 2018, nitrogen was applied three times at all locations as urea via fertigation to achieve an in-season total of 50 kg N ha^{-1} . In 2019, N was applied according to the fertility treatments described previously.

Tomatoes in experimental plots were established on raised beds 12.7 cm (tall) \times 121.9 cm (wide). A water wheel transplanter was used for transplanting, which delivers water as the hole is made in the plastic; this was crucial as the transplanting process required an

adequate amount of moisture around the root ball of the tomato plant. The plants were spaced 152.4 cm apart between rows and 61 cm within the row. The “Florida stake and weave” system were used for trellising the tomato seedlings soon after transplanting into the field. A wooden stake was placed between every other plant in the row (121.9 cm apart), and metal posts were placed every three stakes in the row to provide extra support. Twine was used for training the plants using a figure-eight weave technique, and trellising was done multiple times during the growing season to hold the tomato plants upright and to protect the grafted union from wind injury (Kelley and Boyhan, 2017). At all three locations, the plots were irrigated to field capacity via the drip-irrigation line established underneath the polyethylene mulch. To achieve efficiency for tomato production during the growing season, the on-farm trial at PP applied typical pest control treatments as needed, mainly spraying fungicide and insecticide to control pests like stink bugs (*Halyomorpha halys*), tomato hornworm (*Manduca quinquemaculata*), and anthracnose fruit rot (*Colletotrichum phomoides*). Whereas, organic pesticides such as Bt Worm and Caterpillar killer (Garden Safe®), Copper Fungicide, and Captain Jacks Dead Bug Brew® (Bonide; Oriskany, NY) were applied as needed throughout the growing season at NP and LNK in both years.

Harvesting Procedure

Tomatoes were harvested at a mature pink stage when more than 30% and less than 60% of the fruit surface is showing pink color on both scions (USDA, 1976). Fruits were picked on a weekly basis and started from 67 to 149 days after transplanting (DAT) in 2018 and from 83 to 129 DAT in 2019 at LNK. Fruits were picked every 10 to 14 days from 68 to 132 DAT in 2018 and from 76 to 121 DAT in 2019 at NP. Fruits were picked on a weekly

basis from 70 to 135 DAT in 2018 and 79 to 129 DAT in 2019 at PP. Harvested tomatoes were then graded using USDA standards as marketable (62.5mm to 69mm) or cull (USDA, 1976). In this experiment, cull tomatoes were misshapen, overripe, and seriously damaged by physiological factor such as zippering and bursting, and by bug feeding injury. Fruits in each grade were then counted and weighed fresh. In sync with each harvest interval in 2019, leaf chlorophyll content was estimated for a subsample of plants in each plot using a SPAD-502 Plus Chlorophyll meter (Spectrum Technology Inc., Aurora, IL) (Ling et al., 2011). The readings were taken twice at each location during the growing season, and the early dates were 1 July at LNK, 2 July at NP, and 3 July at PP, whereas the later dates were 15 July at LNK, 17 July at NP, and 23 July at PP.

Statistical Analysis

The experiment was conducted over two years (2018 and 2019), but years were analyzed separately due to differing treatment designs (addition of fertility in 2019). Data within each year were analyzed with ANOVA (proc glimmix; SAS Version 9.4; SAS Institute Inc., Cary, NC) to determine the impact of grafting, location, and nitrogen fertilizer (2019) on tomato yield and leaf greenness (2019). Fixed effects included scion cultivar, rootstock cultivar, location, nitrogen fertility (2019 only), and all possible interactions. The random effect was replicate block. Treatment means were estimated using the LSMEANS statement, and differences among means were determined using the Tukey multiple comparisons test and a significance threshold of $\alpha = 0.05$.

Results and Discussion

Fruit yield in 2018

Results from 2018 indicate that grafting ‘BHN-589’ and ‘Nebraska Wedding’ scions with ‘Maxifort’ and ‘Estamino’ rootstocks did not provide a consistent yield benefit when measured at three locations across Nebraska. Marketable yields were influenced by scion ($P < 0.0001$), rootstock ($P = 0.0042$), and location ($P < 0.0001$; Table 1.3). However, the total yield was not affected by rootstock but significantly influenced by scion ($P < 0.0001$) and location ($P = 0.0098$; Table 1.3). There was no three-way interaction among treatments in either marketable ($P = 0.2070$) or total yield ($P = 0.1557$); however, there was a 2-way interaction between rootstock and location on both marketable ($P = 0.0166$) and total ($P = 0.0103$) yield (Table 1.3).

Despite a significant two-way interaction between rootstock and location ($P = 0.0103$), when averaged over scions, there were no differences in total yield between grafted and nongrafted plants in any of the three locations. Similarly, there were no differences in marketable yield between grafted and nongrafted plants at NP and PP; however, the marketable yield was increased by an average of 48% by nongrafted rootstock when data were pooled across scions at LNK (Figure 1.2). These results supported our findings on percentage marketability, where nongrafted rootstocks produced a higher percentage of marketable fruit compared to other rootstocks when grafted with ‘BHN-589’ ($P = 0.0305$; Table 1.3). Although grafting did not drive differences in yield between the rootstocks, ‘BHN-589’ out-yielded the local heirloom ‘Nebraska Wedding’ by 248% at LNK, 115% at PP, and 94% at NP.

The local favorite hybrid, ‘BHN-589’ is popular among growers for a reason. Not only can it provide a large fruit size and quantity, but it is also notable for conferring resistance to multiple soilborne diseases such as root-knot nematodes, fusarium wilt race 1 and 2, and verticillium wilt (Loewen, 2018; Rivard and Louws, 2011). Multiple studies have reported ‘BHN-589’ to be highly productive on its own, especially under high tunnel production (Maynard and Bluhm, 2018; Oxley and Rivard, 2015, 2016; Rivard et al., 2014). Although it is not clear to why non-grafted ‘BHN 589’ produced greater marketable yield than ‘Maxifort’ and ‘Estamino’ rootstocks at LNK, we hypothesize that the variability in yield is due to the combination of different growing conditions and management at each location. The growing conditions at all three locations were free from extreme biotic and abiotic stresses, which may explain the lack of yield improvement from grafted rootstocks for both scions. Both Djidonou et al. (2020) and Dia et al. (2016) attributed yield differences among grafted plants to variation in local growing conditions. Djidonou et al. (2020) observed a significant interaction between grafting treatment and the environment in both field and high tunnel trials. They stated that most of their environmental variation was from different production systems at each location. We do not expect that soil nutrient levels greatly influenced our results because we did not see large differences in soil nutrients between years at each location (Table 1.1).

All three treatment factors significantly influenced the number of marketable fruits. Total fruits were significantly influenced by scion and location treatments and not the rootstock treatment (Table 1.3). Nonetheless, there were no differences in the number of marketable and total fruits between grafted and nongrafted plants in any of the three locations, except for the number of marketable fruits at LNK. Consistent with fruit weight results at LNK,

BHN-NON produced 54% more fruits compared to BHN-MAX and BHN-EST. As expected, when data were pooled over rootstocks, 'BHN-589' outnumbered 'Nebraska Wedding' on the total number of fruit by an average of 155% at LNK, 28% at NP, and 91% at PP. It is important to note that the fruit size was not influenced by rootstock; however, it differed among scions ($P < 0.0001$) and between locations ($P = 0.0015$; Table 1.3). The fruit size was greatest in LNK (267.32 ± 9.9112 g/fruit), followed by NP (221.84 ± 9.9112 g/fruit) and PP (172.69 ± 9.9112 g/fruit, Table 1.2). Similarly, when data were pooled over rootstocks, 'BHN-589' produced tomatoes that were 38% (LNK) and 37% (NP) larger than 'Nebraska Wedding.' There was no difference in fruit size between scions at PP.

Buller et al. (2013) and Masterson et al. (2016) reported similar results, where grafting did not improve the yield and number of fruits. Both studies were looking at the impact of grafting on heirloom 'Cherokee Purple' with 'Maxifort' under field production. Buller et al. (2013) concluded that the lack of Verticillium wilt incident on the field had limited the benefits of grafting 'Maxifort' with 'Cherokee Purple' in the Pacific Northwest. Whereas 'Masterson et al. (2016) concluded that the heirloom interaction with the rootstock cultivar, plant density, and environment might cause the differences in yield at Reno County in Kansas. Our study is the first published report on yield performance of an old Great Plains heirloom, 'Nebraska Wedding.' When 'Nebraska Wedding' was grafted to 'Maxifort' and 'Estamino' rootstock, the grafted plants performed similarly to nongrafted plants at all three locations.' Perhaps future research should explore additional rootstock combinations when 'Nebraska Wedding' is used as a scion. In contrast to our results, Rivard and Louws (2008) observed an increase in tomato yield when an heirloom 'German Johnson' was

grafted onto ‘Maxifort.’ The study was evaluating the impact of grafting to manage soilborne diseases in heirloom tomato production in North Carolina. Rivard and Louws (2008) emphasized the need for grafting desired scion cultivar with resistant rootstocks, especially in an infested field. ‘Nebraska Wedding’ is a semi determinate cultivar and has a longer growing season with the first harvest date >90 days after transplant (Ozores-Hampton et al. 2003). Perhaps grafting benefits could be realized if ‘Nebraska Wedding’ were grown under a high tunnel system where frost kill can be delayed, and a longer growing season can be obtained.

Fruit yield in 2019

Similar to the results from 2018, grafting both ‘BHN-589’ and ‘Nebraska Wedding’ scions with ‘Maxifort’ and ‘Estamino’ rootstocks in 2019 did not provide a consistent yield benefit when measured at three locations across Nebraska. Marketable and total yields were influenced by scions, fertilizers, and locations ($P < 0.05$), with the effect of rootstocks on total yield approaching significance ($P = 0.0693$; Table 1.5). There was a significant two-way interaction between rootstock and scion on total yield ($P = 0.0045$) and a significant two-way interaction between rootstocks and locations on the marketable yield that was approaching significance ($P = 0.0931$, Table 1.5).

When averaged over scion and fertility treatment, there were no differences in marketable yields between rootstocks within each location (Figure 1.3). However, when averaged over location and fertility treatments, ‘BHN-589’ grafted to ‘Maxifort’ increased total yield by 24% compared to BHN-NON (Figure 1.4). Nonetheless, there were no differences in total yield between rootstocks when grafted to the heirloom ‘Nebraska Wedding’ (Figure 1.4).

Although grafting did not provide a consistent yield benefit between the rootstocks, ‘BHN-589’ out yielded ‘Nebraska Wedding’ by 137% in LNK, 71% in PP, and 83% in NP.

There were no differences in the number of marketable and total fruits between grafted and nongrafted plants among the three locations. As expected, when data were averaged over rootstock and fertilizer treatment, ‘BHN 589’ outnumbered ‘Nebraska Wedding’ on the total number of fruit by 112% in LNK, 72% in NP, and 60% in PP. It is important to note that the average total fruit weight was not influenced by rootstock ($P = 0.5546$) nor fertilizer ($P=0.9204$); however, the average total fruit weight differed among scion ($P = 0.0023$) and between location ($P = 0.0030$; Table 1.5). Surprisingly, in 2019 the average fruit weight in North Platte was 13% higher than the other two locations, and as expected, ‘BHN-589’ average fruit weight was 8% higher than ‘Nebraska Wedding.’

The rootstock ‘Maxifort’ is well known for its ability to improve plant performance by providing scion with the vigorous and resistant rootstock. Although data were not recorded for plant growth purposes, ‘Maxifort’ provided vigorous aboveground growth compared to nongrafted plants (Buller et al., 2013; Hu, 2016; Loewen, 2018; Masterson et al., 2016; Meyer, 2016). Additionally, Loewen (2018) reported that the BHN-MAX interaction was ‘highly compatible’ and consistently provided 40% more yield than the nongrafted ‘BHN-589’. Numerous studies have reported increases in tomato yield and plant growth when ‘Maxifort’ was used as a rootstock (Kunwar et al. 2015; Lang, 2019; Loewen, 2018; Masterson et al. 2016; Meyer, 2016; Rivard and Louws, 2008).

The yield performance of ‘Maxifort’ in this study was not consistent across time or locations. We only observed a single significant yield improvement in both years at all

three locations. Again, we suspected the variability in yield performance is due to the combination of different growing conditions and management at each location. The majority of the studies in the Midwest that observed increases in yield with scion grafted onto ‘Maxifort’ were grown under high tunnel production (Lang, 2019; Loewen, 2018; Masterson et al. 2016; Meyer, 2016). Perhaps this study will encourage future research to explore the impact of grafting vastly under different growing systems and environments, especially in Nebraska.

Hu (2016) reported similar results, where grafting did not provide consistent yield benefits in two consecutive years. Hu (2016) studied the impact of grafting and fertilization on tomato growth, yield, and fruit quality. They observed larger total and marketable fruits number when ‘BHN-589’ were grafted onto ‘Maxifort’ in 2013 but not in 2014 under field production in Wooster, Ohio. Hu (2016) concluded that the difference in yield between those two years was due to unstandardized relative ages of plants.

In regards to ‘Estamino’ rootstock, there was no significant improvement in fruit yield and fruit numbers at all three locations in both years. It is important to note that ‘Estamino’ rootstock has not been extensively studied, but a couple of studies had observed an increase in yield under both high tunnel (Buajaila, 2018; Djidonou et al. 2020; Lang, 2019) and field production systems (Djidonou et al. 2020). Lang (2019) observed an 86% increase in total marketable fruits when ‘BHN-589’ were grafted to ‘Estamino’ and believed the rootstock has a high potential at improving yield under high tunnel production. Meanwhile, Miles et al. (2015) did not observe any significant benefits of grafting ‘Stupice’ onto ‘Estamino’ rootstock on a field infested with *Verticillium* wilt in Washington.

There was no interaction between rootstock and fertility treatment in any of the three locations (Table 1.5). However, when data were pooled across scion and rootstock, the effects of fertility treatment on the total fruit weight were generally significant at LNK and NP, but not the PP location. Plants that received in season N fertilizer ($50 \text{ kg ha}^{-1} \text{ N}$) produced $7.4 \pm 2.5 \text{ kg/plot}$ (LNK) and $16.0 \pm 2.6 \text{ kg/plot}$ (NP) more tomatoes than the control (0 N). Furthermore, the SPAD reading at the earlier date was significantly influenced by scion, location, and fertility treatment, but not the rootstock treatment. In contrast, SPAD reading on a later date was significantly influenced by all four treatment factors (Table 1.7). The estimated leaf chlorophyll content taken on the earlier date ranged between 49.23 ± 2.67 (NW-NON, Zero N) and 60.35 ± 2.67 (BHN-NON, Full N) in LNK, 28.20 ± 2.67 (NW-NON, Zero N) and 51.78 ± 2.67 (BHN-EST, Full N) in NP, and 46.63 ± 2.67 (NW-MAX, Full N) and 58.18 ± 2.67 (BHN-EST, Zero N) in PP (Table 1.6). Comparatively, the estimated leaf chlorophyll content taken on the later date ranged between 45.40 ± 1.70 (NW-MAX, Zero N) and 59.53 ± 1.70 (BHN-NON, Full N) in LNK, 51.61 ± 1.70 (NW-NON, Zero N) and 66.93 ± 1.70 (BHN-NON, Full N) in NP, and 46.45 ± 1.70 (NW-NON, Zero N) and 60.53 ± 1.70 (BHN-NON, Full N) in PP (Table 1.6). Similarly, when data were pooled across scion and rootstock, the effects of fertility treatment on estimated leaf chlorophyll content taken on the later date were generally significant at LNK, and NP, except the PP location. Again, plants that received extra input of N fertilizer (Full N) generated 2.8 ± 0.9 units (LNK) and 3.4 ± 1.0 units (NP) higher than the control (0 N).

Hu (2016) also reported no interaction between rootstock and fertility treatment and had observed increases in yield under high fertility treatment with little to no difference

between the grafted and nongrafted plants. Comparably, Djidonou et al. (2013) also reported an insignificant interaction between rootstock and fertility treatment. However, Djidonou et al. (2013) observed a yield trend that suggested grafted plants produced more yield than nongrafted plants when nitrogen fertilizer rate increased. Leonardi and Giuffrida (2006) studied the impact of grafting on macronutrient uptake and observed the variability of nutrient uptake between different grafting treatments. They concluded that the rootstock phenotype played an important role in macronutrient absorption capacity. In this study, the effect of fertility treatment was only studied by evaluating estimated leaf chlorophyll content and tomato yield performance, which may not have captured exact benefits of grafted plants.

Conclusion

Grafting is known for its potential at suppressing biotic and abiotic stresses in tomato production. With the grafting technique gaining interest among local growers in the Midwest, it is important to provide local growers with the information needed to produce grafted tomatoes under field production here in Nebraska. Given the information that we had gathered in this study, grafting ‘BHN-589’ and ‘Nebraska Wedding’ with ‘Estamino’ and ‘Maxifort’ did not provide a consistent yield improvement under three different field productions across Nebraska. Furthermore, there is no interaction between rootstock and fertility treatment in any of the three locations. We believed grafting had the potential to improve tomato yield under a few circumstances. When a local heirloom ‘Nebraska Wedding’ was grafted onto ‘Estamino’ and ‘Maxifort,’ we had observed similar yield performance with non-grafted plants. We hope future study will perform a different scion-rootstock interaction with ‘Nebraska Wedding’ in an effort to find a great rootstock that will potentially improve the heirloom performance.

Furthermore, grafted plant performance under field study often has three-way interaction between scion, rootstock, and environment. Djidonou et al., (2020) had reported that environmental components contributed up to 86% variation in yield when grafted tomatoes were grown under field conditions. Perhaps, a future study on grafted tomatoes in Nebraska can take place in a growing tunnel, as most of the research that took place across the Midwest had observed the benefits of grafting tomatoes under high tunnel production (Hu, 2016; Lang, 2019; and Loewen, 2018; Meyer et al., 2016). We suggested that our local growers perform thorough planning before diving into grafted vegetable production based

on our findings. Grafted plants have a higher cost of production than non-grafted plants in a traditional growing system. Therefore, we suggested that growers carry out a test run on any desired scion-rootstock combination before investing in grafted vegetable production.

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Figure 1.1

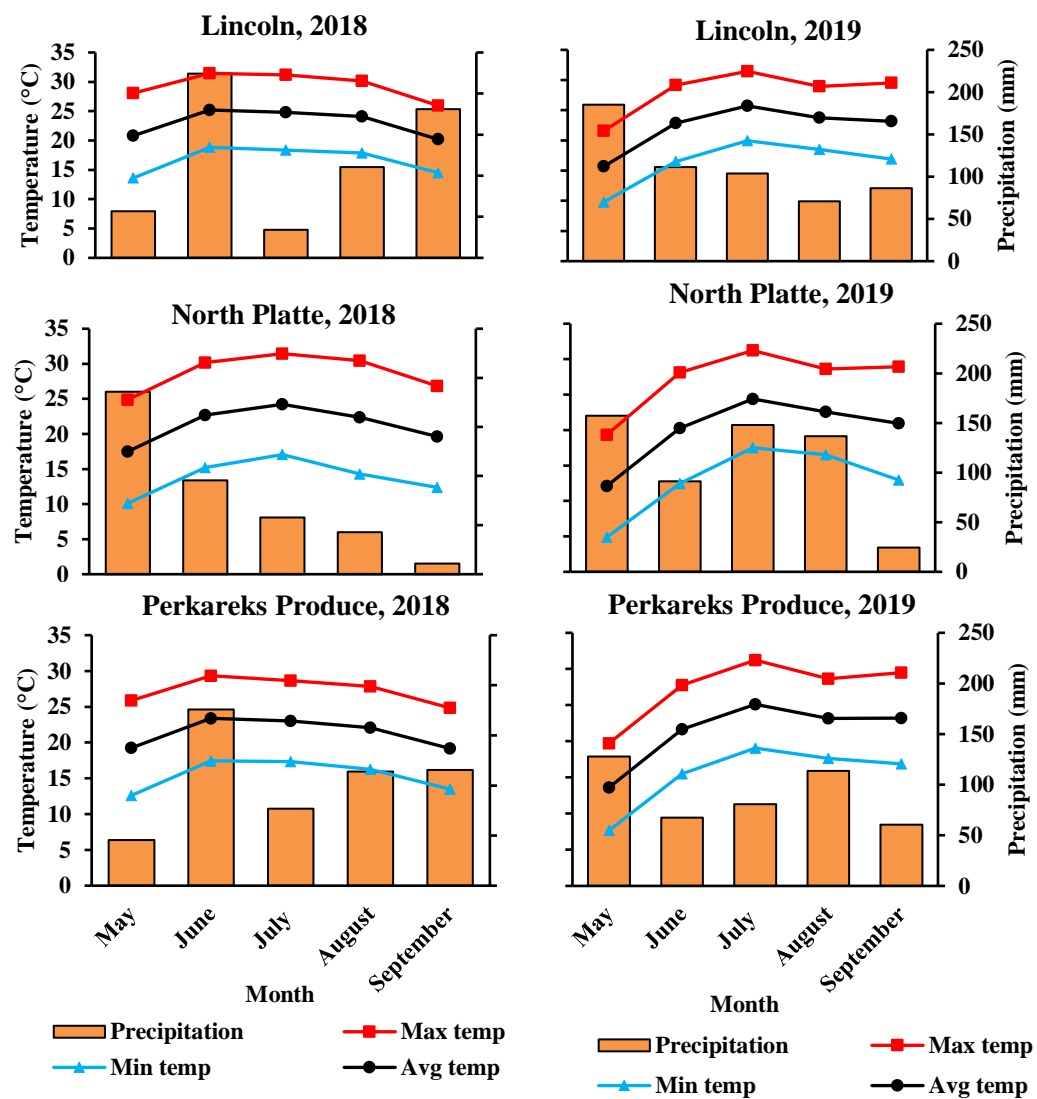


Figure 1.2

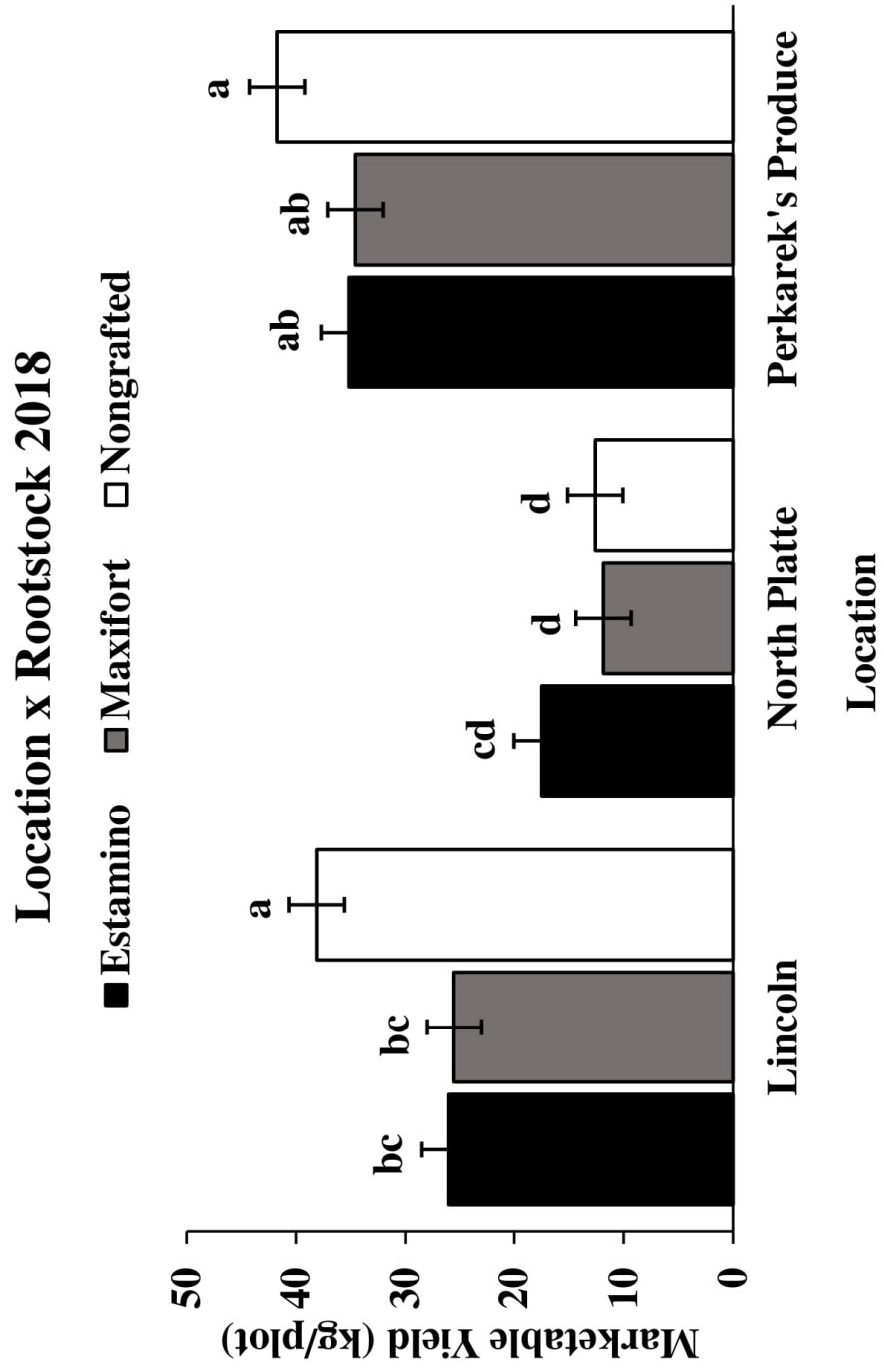


Figure 1.3

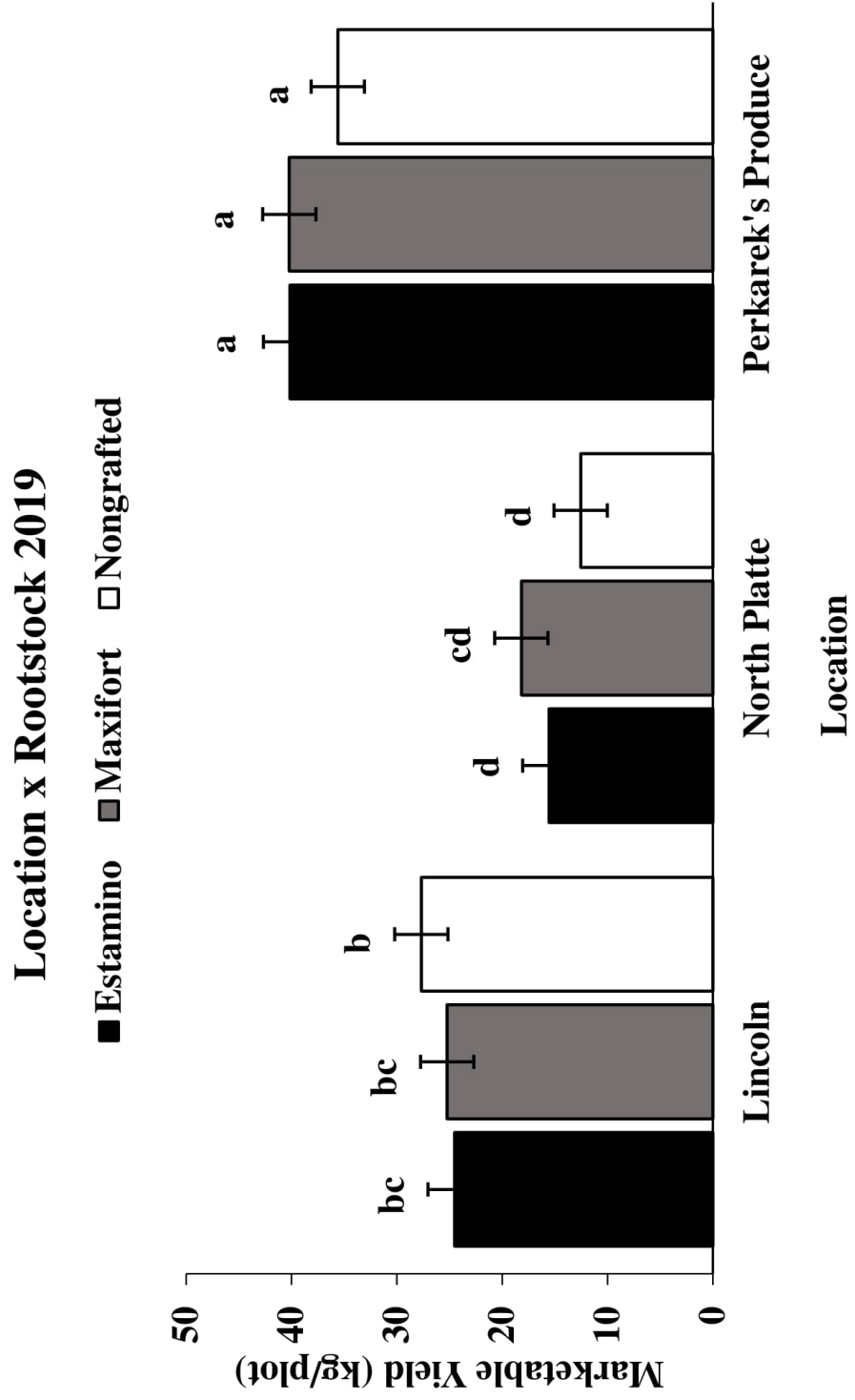


Figure 1.4

Scion x Rootstock 2019

■ Estamino ■ Maxifort □ Nongrafted

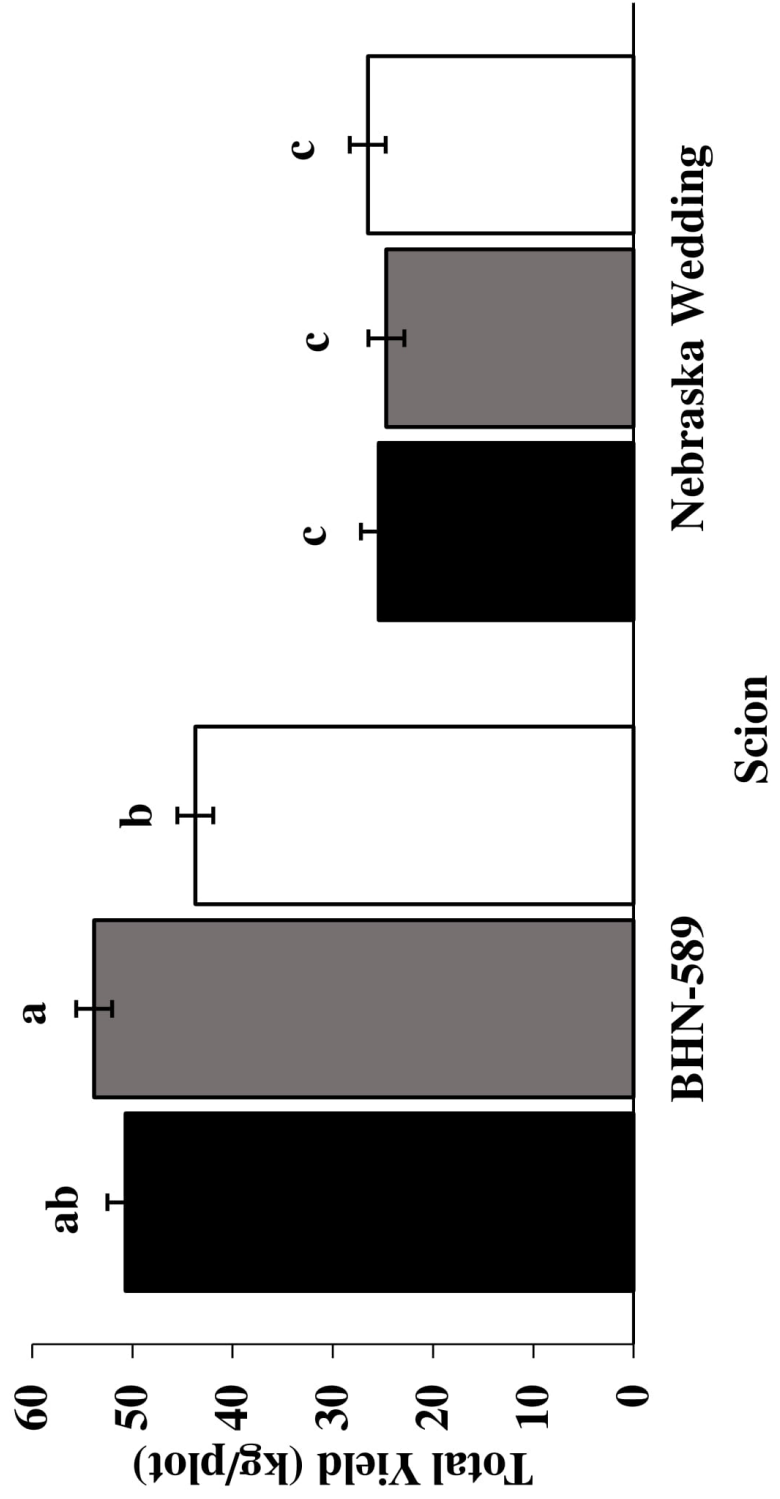


Table 1.1

Location	Year	pH	OM%	CEC (me/100g)	NO ₃ -N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Lincoln, NE (LNK)	2018	6.4	3.6	19.7	9.5	57.0	292.0	2765.0	440.0
	2019	6.6	3.3	18.8	10.9	159.0	519.0	2869.0	361.0
North Platte, NE (NP)	2018	6.8	1.9	8.6	4.7	46.0	456.0	1161.0	194.0
	2019	6.9	2.3	9.8	5.5	84.0	520.0	1641.0	220.0
Dwight, NE (PP)	2018	5.7	3.2	18.6	23.7	10.0	287.0	1671.0	356.0
	2019	5.6	3.7	20.0	27.6	47.0	298.0	2054.0	288.0

Table 1.2

Scion	Rootstock	Marketable Fruit			Total Fruit			Marketability (%)	
		Yield (kg/plot)	No. of fruit	Avg fruit wt (g/fruit)	Yield (kg/plot)	No. of fruit	Avg fruit wt (g/fruit)	Yield	No. of fruit
Lincoln									
BHN 589	Estamino	42.62	132.25	331.46	67.85	215.00	321.29	63.35	61.76
	Maxifort	40.53	125.50	330.42	72.24	228.00	324.41	56.80	54.78
	Nongrafted	59.53	198.25	298.24	81.43	285.50	284.15	73.20	69.42
Nebraska	Estamino	9.41	45.00	239.07	18.31	81.50	233.01	52.34	53.16
Wedding	Maxifort	10.51	45.00	234.68	17.48	78.25	224.45	60.45	57.50
	Nongrafted	16.74	75.00	215.09	27.79	126.25	216.63	58.70	59.42
North Platte									
BHN 589	Estamino	26.29	85.50	286.74	55.80	171.50	317.87	45.98	49.97
	Maxifort	14.97	62.00	236.98	34.98	151.25	231.13	42.96	41.71
	Nongrafted	19.09	84.25	236.16	32.65	154.75	219.74	59.81	55.47
Nebraska	Estamino	8.72	46.50	180.70	24.67	136.00	180.93	34.25	34.02
Wedding	Maxifort	8.73	42.25	208.50	27.93	147.25	189.25	32.52	29.88
	Nongrafted	6.11	32.00	190.49	17.60	90.50	192.11	36.13	35.84
Perkarek's Produce									
BHN 589	Estamino	52.34	279.75	187.30	65.96	369.00	179.30	79.77	76.44
	Maxifort	49.13	250.00	196.72	62.41	335.25	185.94	79.33	74.90
	Nongrafted	53.43	275.00	193.95	60.15	322.00	186.51	88.86	85.41
Nebraska	Estamino	18.02	108.25	166.37	22.33	145.00	154.81	80.82	75.09
Wedding	Maxifort	20.10	112.50	175.73	25.95	162.50	159.78	75.77	69.04
	Nongrafted	30.04	166.75	178.21	39.44	230.75	169.82	75.07	71.61
Standard error		3.57	11.32	27.34	5.55	18.24	24.28	4.64	3.52

Table 1.3

Type III Tests of Fixed Effects	Marketable Fruit			Total Fruit			Marketability (%)	
	Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit
Scion	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Rootstock	0.0042	<.0001	0.6568	0.5735	0.1052	0.3316	0.0177	0.0005
Location	<.0001	<.0001	0.0009	0.0098	0.0001	0.0015	<.0001	<.0001
Scion*Rootstocks	0.2427	0.2514	0.6648	0.1384	0.2948	0.2276	0.0305	0.0579
Location*Scion	<.0001	<.0001	0.0884	<.0001	<.0001	0.0578	0.1456	0.0209
Location*Rootstocks	0.0166	0.0073	0.8769	0.0103	0.0016	0.4150	0.7960	0.8063
Locati*Scion*Rootsto	0.2070	0.0219	0.8315	0.1557	0.0015	0.3862	0.6611	0.5492

Pr > F

Table 1.4

Scion	Rootstock	Fertilizer	Marketable Fruit			Total Fruit			Marketability (%)	
			Yield (kg/plot)	No. of fruit	Avg fruit wt (g/fruit)	Yield (kg/plot)	No. of fruit	Avg fruit wt (g/fruit)	Yield	No. of fruit
Lincoln										
BHN 589	Estamino	Full	43.60	218.75	201.33	61.58	332.25	187.93	70.69	65.92
		Zero	31.35	149.00	211.37	50.15	248.75	202.66	62.15	59.60
	Maxifort	Full	41.13	202.75	202.77	62.38	334.25	186.76	65.72	60.50
		Zero	34.20	172.50	199.00	56.70	272.50	215.22	60.51	63.81
	Nongrafted	Full	45.10	239.25	188.38	60.00	330.25	181.59	75.16	72.42
		Zero	30.98	156.00	198.24	43.23	227.25	190.19	71.46	68.55
Nebraska Wedding	Estamino	Full	12.63	73.25	172.32	20.85	128.00	162.85	60.77	57.31
		Zero	10.48	56.25	190.16	19.65	110.50	178.73	52.58	49.84
	Maxifort	Full	14.38	54.50	264.77	23.15	116.50	205.55	55.37	45.62
		Zero	11.18	63.00	181.61	20.15	124.00	168.66	55.19	51.04
	Nongrafted	Full	18.68	105.25	181.15	31.83	202.00	160.44	58.39	51.73
		Zero	15.95	85.75	186.95	25.55	141.00	181.38	62.68	60.80
North Platte										
BHN 589	Estamino	Full	28.02	128.50	221.16	36.67	166.25	223.59	75.85	76.65
		Zero	11.46	56.00	211.67	18.53	87.75	221.17	57.13	59.18
	Maxifort	Full	28.39	122.00	235.34	38.03	166.75	231.35	74.31	72.99
		Zero	18.91	84.25	223.81	24.91	115.50	214.11	76.71	73.05
	Nongrafted	Full	26.36	115.50	236.58	33.69	149.75	230.15	75.25	73.51
		Zero	9.29	44.50	217.69	14.63	71.50	212.02	60.70	59.03
Nebraska Wedding	Estamino	Full	16.69	80.00	207.86	22.64	107.25	210.93	73.71	74.79
		Zero	5.96	32.00	185.27	8.73	45.75	191.25	67.70	69.80
	Maxifort	Full	18.28	87.50	210.48	25.16	118.25	214.58	71.94	73.38
		Zero	7.16	36.40	194.68	9.90	49.11	198.52	70.33	71.13
	Nongrafted	Full	12.78	58.00	222.14	20.63	98.25	212.94	62.76	60.83
		Zero	1.78	10.00	188.16	3.92	20.97	201.25	35.80	38.26
Perkarek's Produce										
BHN 589	Estamino	Full	51.33	238.00	215.50	66.55	327.75	202.88	77.76	73.17
		Zero	58.53	277.00	211.25	70.73	345.50	205.06	81.68	79.26
	Maxifort	Full	63.68	290.25	218.65	77.48	367.00	210.55	82.30	79.30
		Zero	49.58	238.25	206.32	63.43	322.25	194.53	78.28	73.88
	Nongrafted	Full	45.40	239.00	190.15	57.45	319.75	180.23	78.94	74.84
		Zero	43.73	223.50	194.40	53.35	279.25	190.36	82.02	80.37
Nebraska Wedding	Estamino	Full	26.23	138.25	189.20	37.68	211.75	178.76	69.43	65.66
		Zero	24.48	139.75	176.54	42.90	208.25	203.61	63.75	67.12
	Maxifort	Full	25.20	138.50	182.83	33.85	191.25	177.40	74.59	72.30
		Zero	22.38	122.25	183.34	35.73	201.25	179.00	63.80	62.14
	Nongrafted	Full	29.48	149.50	198.66	42.13	227.00	187.73	70.39	66.77
		Zero	23.83	116.25	204.43	35.08	185.25	189.63	67.86	63.17
Standard error			3.47	16.04	19.79	4.36	21.27	14.61	4.75	4.06

Table 1.5

Type III Tests of Fixed Effects	Marketable Fruit				Total Fruit				Marketability (%)	
	Yield	No. of fruit	Avg fruit weight		Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit	
	Pr > F									
Scion	<.0001	<.0001	0.0308	<.0001	<.0001	<.0001	0.0023	<.0001	<.0001	<.0001
Rootstock	0.2031	0.6803	0.4665	0.0693	0.4840	0.5546	0.4695	0.2328	0.2328	0.2328
Fertilizer	<.0001	<.0001	0.1495	<.0001	<.0001	0.9204	0.0003	0.0003	0.0041	0.0041
Location	<.0001	<.0001	0.1659	<.0001	<.0001	0.0030	0.0070	0.0070	0.0026	0.0026
Scion*Rootstock	0.0709	0.3582	0.5220	0.0045	0.0309	0.6211	0.0838	0.0838	0.0102	0.0102
Location*Scion	<.0001	<.0001	0.5000	<.0001	<.0001	0.8341	0.3207	0.3207	0.0323	0.0323
Location*Rootstock	0.0931	0.0216	0.7146	0.2331	0.2133	0.6822	0.0002	0.0002	<.0001	<.0001
Location*Fertilizer	0.0054	0.0086	0.6199	0.0010	0.0107	0.1451	0.0618	0.0618	0.0024	0.0024
Scion*Fertilizer	0.1117	0.0838	0.3969	0.1143	0.1028	0.7406	0.6538	0.6538	0.9124	0.9124
Rootstock*Fertilizer	0.6353	0.3751	0.4803	0.2790	0.1359	0.4141	0.5033	0.5033	0.4565	0.4565
Scion*Rootst*Fertili	0.9097	0.9302	0.8057	0.9484	0.8178	0.6751	0.8291	0.8291	0.7823	0.7823
Locati*Scion*Rootsto	0.2297	0.7769	0.3719	0.3604	0.5112	0.8694	0.1070	0.1070	0.1225	0.1225
Locati*Scion*Fertili	0.2905	0.0800	0.7025	0.6812	0.1960	0.5056	0.2839	0.2839	0.2160	0.2160
Locati*Rootst*Fertil	0.2488	0.0796	0.4072	0.5504	0.7211	0.9733	0.0177	0.0177	0.0018	0.0018
Loca*Scio*Root*Ferti	0.2140	0.4298	0.6273	0.5076	0.7428	0.3660	0.4326	0.4326	0.2659	0.2659

Table 1.6

Scion	Rootstock	Fertilizer	SPAD reading	
			Earlier date	Later date
Lincoln				
BHN 589	Estamino	Full	58.33	56.30
		Zero	59.35	54.83
	Maxifort	Full	57.23	58.08
		Zero	57.55	52.33
	Nongrafted	Full	60.35	59.53
		Zero	58.13	56.00
Nebraska	Estamino	Full	49.98	49.40
		Zero	50.15	48.35
Wedding	Maxifort	Full	49.25	46.23
		Zero	49.30	45.40
	Nongrafted	Full	53.00	53.55
		Zero	49.23	49.35
North Platte				
BHN 589	Estamino	Full	51.78	59.80
		Zero	37.93	55.78
	Maxifort	Full	50.35	62.60
		Zero	51.60	61.28
	Nongrafted	Full	50.20	66.93
		Zero	35.85	60.70
Nebraska	Estamino	Full	50.68	55.43
		Zero	35.65	52.98
Wedding	Maxifort	Full	50.33	53.70
		Zero	36.46	51.90
	Nongrafted	Full	51.00	56.30
		Zero	28.20	51.61
Perkarek's Produce				
BHN 589	Estamino	Full	56.70	56.35
		Zero	58.18	56.20
	Maxifort	Full	56.05	52.58
		Zero	53.10	55.28
	Nongrafted	Full	61.45	59.73
		Zero	57.75	60.53
Nebraska	Estamino	Full	48.70	49.38
		Zero	49.45	49.85
Wedding	Maxifort	Full	46.63	49.88
		Zero	47.33	49.20
	Nongrafted	Full	49.53	50.38
		Zero	50.98	46.45
Standard error			2.67	1.70

Table 1.7

	SPAD reading	
	Early date	Later date
Type III Tests of Fixed Effects	Pr > F	
Scion	<.0001	<.0001
Rootstock	0.9905	0.0002
Fertilizer	<.0001	0.0002
Location	0.0001	0.0017
Scion*Rootstock	0.7764	0.0274
Location*Scion	0.0881	0.9955
Location*Rootstock	0.0051	0.2643
Location*Fertilizer	<.0001	0.0341
Scion*Fertilizer	0.2189	0.9858
Rootstock*Fertilizer	0.0568	0.156
Scion*Rootst*Fertili	0.7567	0.7039
Locati*Scion*Rootsto	0.6339	0.0086
Locati*Scion*Fertili	0.0381	0.2615
Locati*Rootst*Fertil	0.1382	0.7832
Loca*Scio*Root*Ferti	0.4264	0.6179

Chapter 2 – The Impact of Water and Nitrogen Treatment on Grafted Tomato

Abstract

Growers all around the world have been struggling to improve water and nutrient efficiency in vegetable production. For a variety of ornamental and specialty crops, grafting the desired cultivar with a higher root density rootstock is known to improve water and nutrient uptake efficiency. Such innovative tools can be very beneficial for vegetable production, such as tomatoes. A grafting experiment was conducted under limited water and Nitrogen (N) condition. The greenhouse experiment took place at the University of Nebraska Lincoln - East Campus in Lincoln, Nebraska, from fall 2018 to early summer 2019. Determinant fresh market tomato, 'BHN-589' were grafted onto one of two potentially valuable rootstocks, 'Estamino' and 'Maxifort,' with the nongrafted scion cultivars as controls. Nitrogen fertilizer treatments were implemented at 0.5 X, 1.0 X, and 1.5 X of 120 ppm of N, and water treatment was divided into high (above field capacity) and low (below field capacity). Overall, grafting did not have any consistent impact on fruit yield. 'Estamino' improved % of fruits marketability by 28% compared to the nongrafted plants especially under 1.5 X of N fertility treatment. However, there were no significant difference in total and marketable yield between grafted and nongrafted plants. Moreover, 1.5X N reduced % marketability of the tomatoes under both high and low water treatment.

Introduction

Nitrogen is an essential plant macronutrient and is the main necessary for the formation of proteins responsible for biochemicals, including enzymes, chlorophyll, and nucleic acids in plants (Brady and Weil, 2010; Haruna et al., 2017). It has always been a challenge for farmers and small-scale growers to find the “happy medium” of nitrogen (N) needed for crops each growing season without hurting the plants and the environment (Dinnes et al., 2002). Lack of knowledge about nutrient management results in poor agronomic practices that can lead to nitrate (NO_3^-) leaching, often from over application and ill-timed N fertilizer application (Hallberg, 1989; Hatfield and Cambardella, 2001; Linville and Smith, 1971). It has been reported that between 50% and 70% of N can be leached from intense agronomic production systems (Asgari and Cavagnaro, 2011; Raun and Johnson, 1999). Leaching of NO_3^- into the groundwater represents a loss of available N to plants, degrades groundwater quality, and represents a significant risk to drinking water supplies (Haruna et al., 2017). Because N is such a vital element for plant growth and productivity, but can also contribute to negative environmental impact, growers should strive for improved nitrogen use efficiency (NUE).

Nitrogen fertilizer is essential for tomato (*Solanum lycopersicum*) production as it promotes crop growth and development and is related to uptake of other nutrients (Aczel, 2019). Lowrance and Smittle (1988) suggest that vegetable crop production results in lower NUE than production of agronomic crops such as corn and soybean. Thus, new approaches are needed to improve NUE in vegetable crops like tomato, including better planning and management with mindful consideration for the environment (Angus, 1995; Power and Schepers, 1989).

Another yield-limiting factor in tomato production is water. Limited water resources have always been a great challenge for growers in arid and semi-arid areas. Irrigation water is extensively pumped from groundwater resources, and if continued, will begin to jeopardize long-term water security (Kang et al., 2004). Innovative approaches are needed to improve water use efficiency in all crops, including tomato, that balance crop water needs and long-term sustainability. Irrigation is essential in many vegetable crops, and tomato is particularly sensitive to water-limiting conditions. Yield reduction can be expected if tomato plants are exposed to water deficit even intermittently, especially during the fruit-bearing stage, due to a common physiological disorder in tomato called blossom end rot (BER). BER is a commonly occurring calcium deficiency of tomatoes and early symptoms include small, water soaked spots. As the fruit continues to develop, the spots enlarge and become flattened, black, and leathery. Since calcium is a relatively immobile essential plant nutrient, tomato plants rely on mass flow and transpiration to move calcium to the fruit and leaves. The transpiration rate of the tomato fruit is reduced as the fruit matures and produces a waxy cuticle; therefore, the fruit serves as a poor calcium sink compared to the leaves. Thus, blossom end rot occurs when mass flow and transpiration are low due to reduced soil moisture. Taylor and Locascio (2014) reported that BER may reduce global tomato yield by up to 50%.

Plants grown under undesirable or stressful growing conditions are often more susceptible to disease and infection (Flores et al. 2010; Savvas et al., 2011). Many researchers have recommended grafted plants to combat these stressful growing conditions by improving nitrogen and water use efficiency (Lee and Oda, 2003; Schwarz et al., 2013). Grafting is a vegetative propagation method where the shoot (scion) of one cultivar is combined with

the roots (rootstock) of a second cultivar to create a new hybrid plant. Grafting is an essential non-chemical crop production tool that has been used to combat abiotic stresses among many ornamental and vegetable products (Flores et al. 2010; Savvas et al., 2011). Grafted plants are also popular for combatting soil borne diseases, especially when crop rotation and diversity is limited (Rivard, 2006). Given these benefits, many studies have reported increased plant productivity and fruit yield when grafting a desirable scion cultivar onto a rootstock selected for disease tolerance, vigor, or resource use efficiency (Barrett et al., 2012; Flores et al., 2010; Mc Avoy et al., 2012; Rivard and Louws, 2008; Savvas et al., 2011); and these same yield benefits of grafting in tomato have been observed in multiple greenhouse studies (Khah et al., 2006; Passam et al., 2005; Pogonyi et al., 2005; Soare et al., 2018). Ruiz and Romero (1999) and Lee (1994) both attributed increased yield of grafted plants to enhanced nutrient and water uptake.

Tomato is the most common vegetable crop grown in Nebraska and throughout the Midwest, especially on small-scale diversified farms (USDA NASS, 2020). Given the interest in this crop and the scale of production, it is important to explore the possibility of using grafted tomato plants to improve nitrogen and water management in Nebraska. The objective of this study was to assess the potential for two popular tomato rootstocks ('Estamino' and 'Maxifort') to maintain or increase yield of the popular hybrid 'BHN-589' tomato under water and nitrogen limiting conditions. The study was conducted in a controlled environment greenhouse to enable precise management of nitrogen and water inputs. Given results of similar studies and knowledge of rootstock root properties, we hypothesized that grafted plant yield would be greater than nongrafted yield when managed with reduced or deficit irrigation water and nitrogen levels.

Materials and Methods

Experimental Design

A greenhouse experiment was conducted at the University of Nebraska - Lincoln from fall 2018 to early summer 2019 to explore the potential benefits of grafted tomatoes on water and nitrogen use efficiency and yield. Greenhouse temperature was set between 26.7 to 32.2 °C during the daytime and 15.6 to 21.1 °C during the night. LEDs provided supplemental light for 16 hours per day. The experiment was arranged in a randomized complete block design. It included a $3 \times 2 \times 3$ factorial combination of three rootstock cultivars, two irrigation regimes, and three N fertilizer levels, with six replicate blocks and a total of 18 experimental units (i.e., potted plants) per block (Figure 2.1).

Grafting Treatment

Rootstock cultivars included ‘Maxifort,’ a popular vegetative rootstock hybrid, and ‘Estamino,’ a generative rootstock hybrid (Johnny’s Selected Seeds, Winslow, ME). A local grower-favorite, commercial hybrid, ‘BHN-589,’ was grafted onto the rootstock cultivars, and a nongrafted plant served as a control. The germination and emergence of rootstock seeds took longer than the scion seeds. Therefore, rootstock seeds were sown on 29 Nov. – four days earlier than the scion seeds, which were sown on 3 Dec. This was done to increase the likelihood that the diameter of scion and rootstock seedling stems would be similar to increase the grafting success rate. All seedlings were sown in 72-cell plug trays (each cell was 3.8 cm \times 3.8 cm \times 5.7 cm deep) filled with a soilless potting mix that included coarse grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic

limestone, non-ionic wetting agent, and standard fertilizer starter charge (Berger Mix BM1; JR Johnson, St. Paul, Minnesota). Approximately one month after seeding, on 8 January, ‘BHN-589’ scions were splice-grafted onto ‘Maxifort’ and ‘Estamino’ rootstocks. The grafting work area was sterilized with isopropyl alcohol prior to and during the grafting session.

Grafting Procedure

To begin the grafting procedure, shoots of the rootstocks were first removed below the cotyledons at a 45° angle using a miter-cut grafting knife (Johnny’s Selected Seeds, Winslow, ME). Next, an identical 45° angle cut was made of the scions using the same knife. The scion and rootstock stems were carefully joined together and secured with a 1.5 mm diameter silicon tube (Johnny’s Selected Seeds, Winslow, ME). After grafting, plants were immediately transferred into a closed healing chamber built of polyvinyl chloride pipe and clear polyethylene plastic. Chambers were equipped with a thermometer, two humidifiers, and a relative humidity sensor. Light transmission was filtered from the chamber using white linens for the first seven days after the grafted tomatoes were transferred into the chamber. Additional layers of linen were removed on a daily basis after seven days to allow the grafted tomatoes to acclimate to ambient greenhouse conditions. Temperature and the average relative humidity of the healing chamber were maintained between 21 to 27 °C and >90% humidity for the first three days. On 11 Jan., the humidity level was reduced to 70%. Ten days later, relative humidity was reduced by opening up the chamber and increasing light exposure. Grafted plants were watered gently at the base of the plants as needed, and adventitious roots were removed with pruning shears or razor

blade as needed. On 24 Jan., plants were moved from the healing chamber and into the greenhouse alongside the nongrafted seedlings. Thereafter, all plants were fertigated with a 20N-10P-20K fertilizer solution on a weekly basis until transplanted into larger pots on 8 Feb.

Greenhouse Setup

Black polyethylene pots (30.2 cm in top diameter, 28 cm in-depth, and a volume of 18.9 L) were filled with a soilless potting mix (Berger Mix BM1; JR Johnson, St. Paul, Minnesota). Pots were watered prior to the transplanting process and beneath each pot was a leach tray (28 cm × 53.3 cm × 5.7 cm) intended to prevent loss of nutrients leached from the bottom of pots during irrigation. The plants were 53.3 cm apart in each row and 28 cm apart between the rows (Figure 2.1). Each pot was transplanted with one tomato seedling, and the “Florida stake and weave system” – typically used in field fresh market tomato production systems – was adapted to trellis the tomato plants on 1 Mar., which was 20 days after plants were transplanted into the pots. A bamboo stake was placed in every pot, and wooden posts (secured in a 19.4 L bucket of concrete) were placed every seven to eight pots within each row to provide extra support. Twine was used to train the plants using a figure-eight weave technique, and trellising was done multiple times during the growing season to hold the tomato plants upright and to protect the grafted union from mechanical injury.

Water Treatment

Water treatments were imposed on 22 Feb., and prior to that, all plants were hand-watered to meet visually approximated evapotranspiration (ET) demand. An automated irrigation

system was constructed to deliver prescribed water treatments (high and low) at a scheduled time in all 108 pots. Pots in the ‘high’ water treatment received enough water to achieve field capacity (determined as water dripping from the bottom of the pot) and pots in the ‘low’ water treatment were managed with a deficit irrigation approach with a target of between 43% and 67% of the volume applied in the ‘high’ treatment (Table 2.1). The exact irrigation volume on each day was determined based on visual assessment of soilless media moisture and plant health to avoid extreme moisture stress. A 2-outlet digital timer (Orbit, North Salt Lake, UT) was attached to the faucet, and the water pressure was set at 10 - 13 psi. A black polyvinyl chloride tube was used to connect the outlet with individual “dribble rings” (Dramm Corp., Manitowoc, WI, USA). Each dribble ring was placed in each pot to try and achieve uniform irrigation across the surface of the pot (Nemali and van Iersel, 2006; Figure 2.2 and 2.3). Pots were watered on a daily basis after 20 Mar. when plants had started the flowering stage. Consequently, as the tomato entered the fruit development stage, the volume of water received in each treatment was increased. Irrigation frequency increased to twice per day when tomato fruit began showing blossom end rot symptoms on 4 Apr. (Table 2.1).

Fertilizer Treatment

Tomatoes received one of the three fertilizer treatments, including 0.5X, 1.0X, and 1.5X N. The 1.0X N treatment application rate was determined based on a Continuous Liquid Feed (CLF) recommendation of supplying 120 ppm of N and 220 ppm of calcium per plant. Thus, the 0.5X N treatment received 60 ppm of N, and the 1.5X N treatment received 180 ppm of N. Fertigation solution was prepared with Peter’s Professional (5N-11P-26K; ICL

Fertilizers, Dublin, OH), calcium nitrate (15.5N-0P-0K+26.5 CaO; Hummert International, Topeka, KS), and urea (46N-0P-0K; Howard Johnson's Enterprises, Inc., Franklin, WI). On 2 Apr., liquid limestone (0N-0P-0K+25.1 Ca; Burnett Lime Company, Inc., Campobello, SC) was added to the fertigation solution to alleviate symptoms of blossom end rot. Each pot received one liter of the fertigation solution via hand fertigation. Fertilizer treatment began on 19 Feb. and ended on 2 July (Table 2.1). Tomato plants received a total of 36 fertigation treatments throughout the growing period (Table 2.1).

Harvesting Procedure

Tomato harvesting began on 9 Apr. and ended on 9 July. Tomatoes were harvested on a weekly basis and at a mature pink stage, when more than 30% but not more than 60% of the fruit surface (across all treatments), in the aggregate, showed pink color (USDA, 1997). Harvested tomatoes were then graded as marketable or cull (cracked, damaged, and diseased). All marketable fruits met a minimum criteria of the "U.S. No. 2" grade for fresh tomato production (USDA, 1997). Fruits in each category were then counted and weighed fresh.

Statistical Analysis

Yield data was analyzed with ANOVA (proc glimmix; SAS Version 9.4; SAS Institute Inc., Cary, NC) to determine the impact of grafting, irrigation regime, and N fertilizer on tomato yield. Fixed effects in the generalized linear mixed effects model included rootstock, irrigation regime, N fertilizer, and all possible interactions of these factors. The random effect was replicate block. Treatment means were estimated using the LSMEANS

statement, and differences among means were determined using the Tukey multiple comparisons test and a significance threshold of $\alpha = 0.05$.

Results and Discussion

Total fruit yield

There were no differences in total yield or total number of fruit among grafted rootstocks and the nongrafted plants. However, total yield was influenced by irrigation regime ($P < 0.0001$), and the effect of N treatment was approaching significance ($P = 0.0806$; Table 2.3). The total number of fruit was not affected by rootstock or irrigation regime, but was influenced by N treatment ($P = 0.0211$; Table 2.3). Total yield ranged between 6.7 ± 0.5 kg/plant (nongrafted - high water - 1.0X N) and 4.1 ± 0.5 kg/plant ('Estamino' rootstock - low water - 1.5X N, Table 2.2).

Previous studies on grafted tomato plants in controlled environments often reported improved plant performance in grafted compared to nongrafted plants. These results include higher average fruit weight (Pogonyi et al., 2005), a higher number of fruits per truss (Ibrahim et al., 2001), and greater total fruit yield (Khah et al., 2006; Marsic and Osvold, 2004; Soare et al., 2018; Turhan et al., 2011) as compared to nongrafted plants. However, grafting 'BHN 589' to 'Maxifort' and 'Estamino' rootstocks conferred no benefits in our study. It is hypothesized that the primary mechanism for improved resource use efficiency in grafted plants is increased root surface area of the rootstocks (Lee and Oda, 2003). However, the 18.9 L plastic pots used in this study and limited root volume may have mitigated any potential benefit of a vigorous rootstock with greater root surface area. Oztekin et al. (2009) assessed the root characteristics of vigor rootstocks such as

‘Heman’ and ‘Beaufort’ as compared to the cultivar ‘Durinta.’ In one of the assessments, grafted plants grown in a 10 L pot had a similar root length compared to nongrafted plants. Moreover, the ‘BHN-589’ cultivar used in this study is known for its resistance to multiple soil-borne diseases (Loewen, 2018; Rivard and Louws, 2011) and its high productivity (even in the absence of grafting) (Maynard and Bluhm, 2018; Oxley and Rivard, 2015, 2016; Rivard et al., 2014).

Soylemez and Pakyurek (2017) and Borgognone et al., (2012) reported similar results, where total yield and total number of fruits were not affected by rootstock-scion combination. Borgogne et al., (2012) studied the impact of nitrogen form (ammonium versus nitrate) on grafted tomato growth in a greenhouse hydroponic system and failed to observe any differences in yield when ‘Moneymaker’ was grafted onto ‘Maxifort,’ and concluded that yield was highly influenced by the nitrogen form rather than the grafting treatment. Soylemez and Pakyurek (2017) studied the impact of different electrical conductivity (EC) levels and grafting on tomato plant performance in a greenhouse environment. Soylemez and Pakyurek (2017) observed little to no difference in yield when ‘Newton’ was grafted onto ‘Maxifort’ and ten other commercial rootstocks. They found that increasing the EC level reduced the total and marketable yield in both years and that tomato yield was influenced by different nutrient solution EC levels, but not rootstocks.

The average individual fruit weight was significantly affected by irrigation and N treatment ($P < 0.0001$; Table 2.3) and ranged from 150.5 ± 7.4 g/fruit (‘Estamino’ - high water - 0.5X N) and 85.6 ± 7.4 g/fruit (‘Maxifort’ – low water - 1.5X N; Table 2.2). The interaction between N and water was approaching significance ($P = 0.0697$; Table 2.3). When data were pooled across rootstocks, average fruit weight decreased by >34% as the N input

increased, especially under water-limiting conditions. Du et al (2017) observed similar results as applications of $> 250 \text{ kg ha}^{-1}$ N paired with limited water availability reduced average fruit weight in a greenhouse environment.

Marketable fruit yield

There were no differences in marketable yield among grafted rootstocks and nongrafted plants. Marketable yield was not affected by rootstock, but was significantly influenced by water and N treatment ($P < 0.0001$; Table 2.3). In addition to the results of Borgognone et al. (2012) and Soylemez and Pakyurek (2017) discussed previously, Lang (2019) also found no effect of rootstock on marketable yield when ‘Mountain Fresh Plus’ and ‘Cherokee Purple’ were grafted onto ‘RST-04-106-T’ in a high tunnel greenhouse environment. Lang (2019) concluded that the lack of soil-borne pathogens in the high tunnel was cause for the lack of yield differences. This, combined with our results, suggests that the use of grafted tomato plants should be limited to cases where soilborne disease has been documented because changes in resource use efficiency are less consistent.

Regardless of the rootstock treatment, marketable yield in the 1.5X N treatment was reduced by an average of 31% when combined with low water treatment. Numerous studies have emphasized the importance of the synergistic effect of water and nutrient input on yield performance (Akemo et al., 2000; Djidonou et al., 2013; Du et al., 2017; Topçu et al., 2007; Wang and Xing, 2017). Nitrogen is an essential plant nutrient, but excessive input of N in tomato can reduce yield due to overly vigorous vegetative growth and reduced flower formation and fruit set (Kaniszewski and Elkner, 1990; Abu-Alrub et al. 2019).

All treatment factors influenced % marketability of tomato fruit (Table 2.3). There were significant interactions between water and N treatment ($P = 0.0045$), and the interaction

between N fertilizer and rootstock was approaching significance ($P = 0.0559$; Table 2.3). When pooled across water treatments, grafting ‘BHN-589’ to ‘Estamino’ at the 1.5X N fertilizer level helped to improve fruit marketability by 28% compared to the nongrafted control (Figure 2.4). Several studies on ‘Estamino’ have reported an increase in yield under both protected environment (Buajaila, 2018; Djidonou et al. 2020; Lang, 2019) and field production systems (Djidonou et al. 2020). Lang (2019) observed an 86% increase in total marketable fruits when ‘BHN-589’ was grafted to ‘Estamino’ in a high tunnel production system. Meanwhile, Miles et al. (2015) did not observe any significant benefits of grafting ‘Stupice’ onto an ‘Estamino’ rootstock in a field infested with *Verticillium* wilt in Washington.

Across all rootstocks, tomatoes that received the highest fertilizer rate reduced fruit marketability by 32% under the low water treatment, and by 22% under the high water treatment when compared with the other fertilizer treatments (Figure 2.5). In order to achieve a better yield and save costs on fertilizer input, an optimum supply of N and water is needed (Gebremariam and Tesfay, 2019). Du et al. (2017) found that high nitrogen inputs resulted in low nitrogen use efficiency. When the N fertilizer rate is beyond optimum, nitrogen is accumulated in storage organs or lost to the environment and does not contribute to increased yield or production efficiency (Song et al., 2009; Min et al., 2011; Du et al., 2017).

Cull fruit yield

Both rootstock treatment and N treatment affected cull yield ($P < 0.05$; Table 2.3). There were significant interactions between N and rootstock treatment ($P = 0.02$), as well as N and water treatment ($P = 0.028$; Table 2.3). When averaging over water treatment, grafting

'BHN-589' scion with 'Estamino' significantly reduced cull fruits by 25% under 1.0X N and by 32% under 1.5X N, as compared to the other two rootstocks (Figure 2.6). Similar to observations of % marketability, grafting 'BHN-589' with 'Estamino' may result in improved quality and marketability of fruit produced.

When averaged across rootstocks, the 1.5X N fertilizer treatment produced almost five times more cull tomatoes as compared with 0.5X fertilizer treatment under water-limiting conditions. The vast majority of these tomato fruit were culled due to the presence of BER, which is a physiological disorder related to calcium deficiency and drought stress. We observed BER symptoms beginning 4 Apr. and started fertigation with liquid limestone at that time. However, we continued to observe greater cull yield due to BER, especially in the low water treatment and the 1.5X N input. Both Gebremariam and Tesfay (2019) and Warner et al. (2004) reported increases in BER incidents as the N fertilizer rate increased. Blossom end rot occurs mostly under limited water conditions due to calcium relative immobility in plants and soil. Therefore, tomato plants often rely on mass flow and transpiration to move calcium to the fruits and leaves (Taylor and Locascio, 2004). Thus, high N fertilizer rates could have inhibited calcium uptake by plants (Vitousek et al., 2009), and the negative effects were likely compounded under water-limiting conditions of the low water treatment.

Conclusion

While grafting had proven to improve water and nutrient uptake, in this study we barely observed any significant impact that differentiate the total and marketable yield between grafted and nongrafted plants. However, 'Estamino' had shown a significant reduced in cull yield and a significant increase in % marketability compared to 'Maxifort' and nongrafted 'BHN-589.' We believe grafting will provide beneficial trait if given the right condition. We suspected the 18.9 L pot used in this study had limited the potential of grafting on providing higher marketable and total yield. It is important to note that this greenhouse study only looked at one growing season and only have six replications in total. Perhaps, these explained part of the reason why we did not see a huge different in yield between grafted and nongrafted plant. Providing the information that we had gathered on this study, we suggested growers to do a test run on 'Estamino' and 'Maxifort' prior to growing the plants for production purposes. Regardless of the rootstocks, we had observed a decrease in yield when higher than 120 ppm of Nitrogen is used under both water treatments. Since vegetable grafting production itself requires a higher total investment, growers should save up on N fertilizer and apply to the crop if needed.

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Figure 2.1

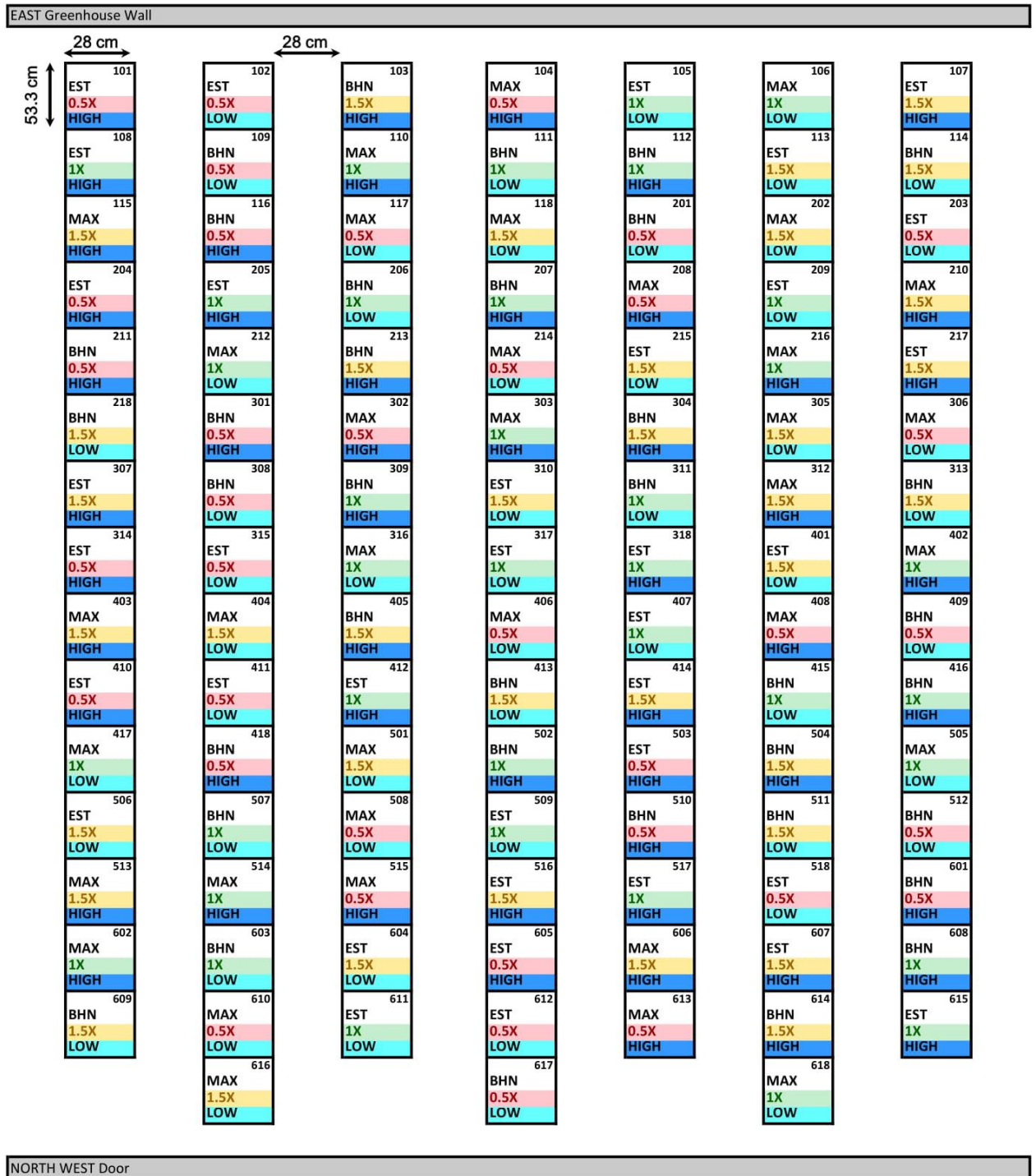


Figure 2.2



Figure 2.3



Figure 2.4

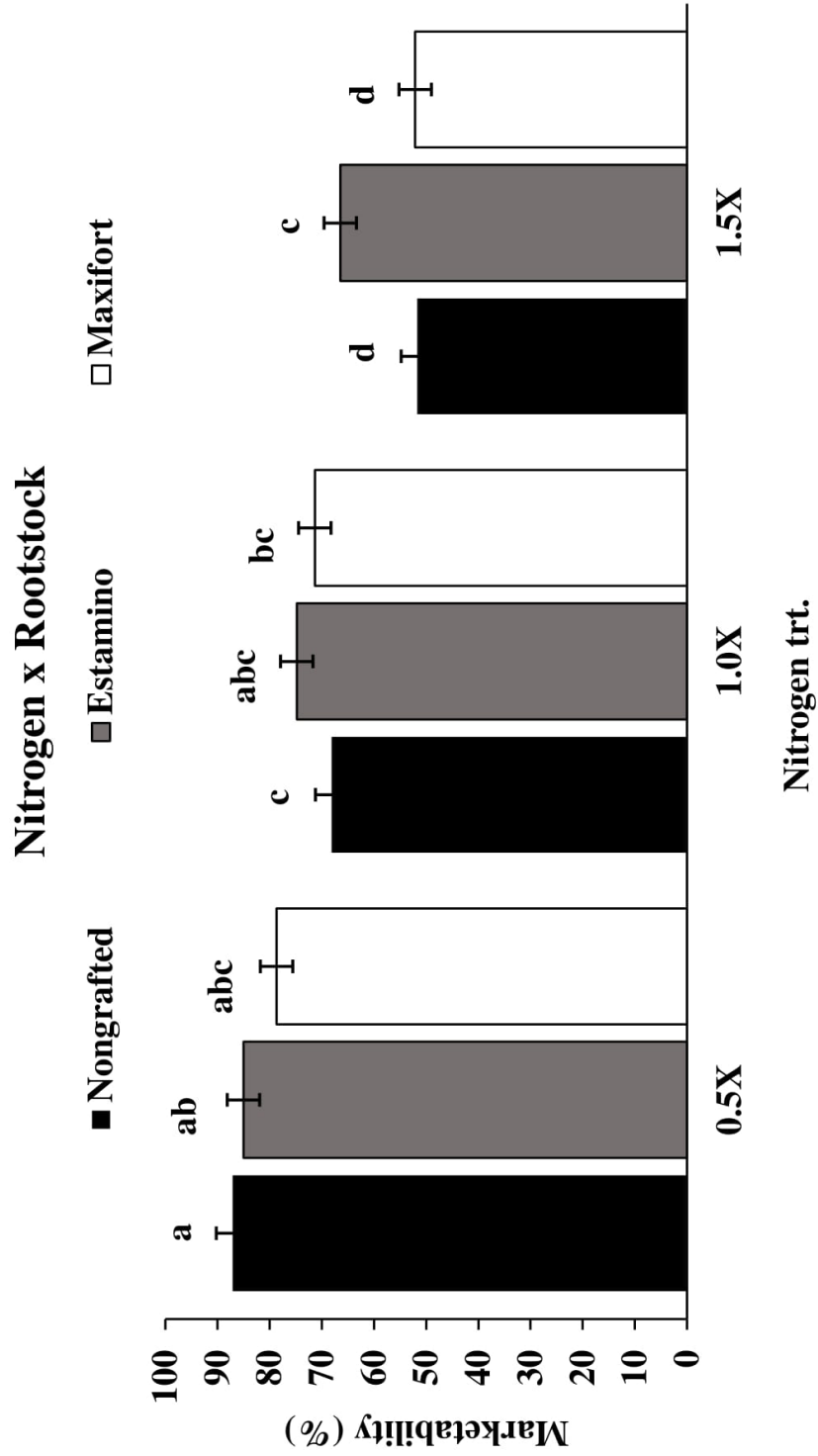


Figure 2.5

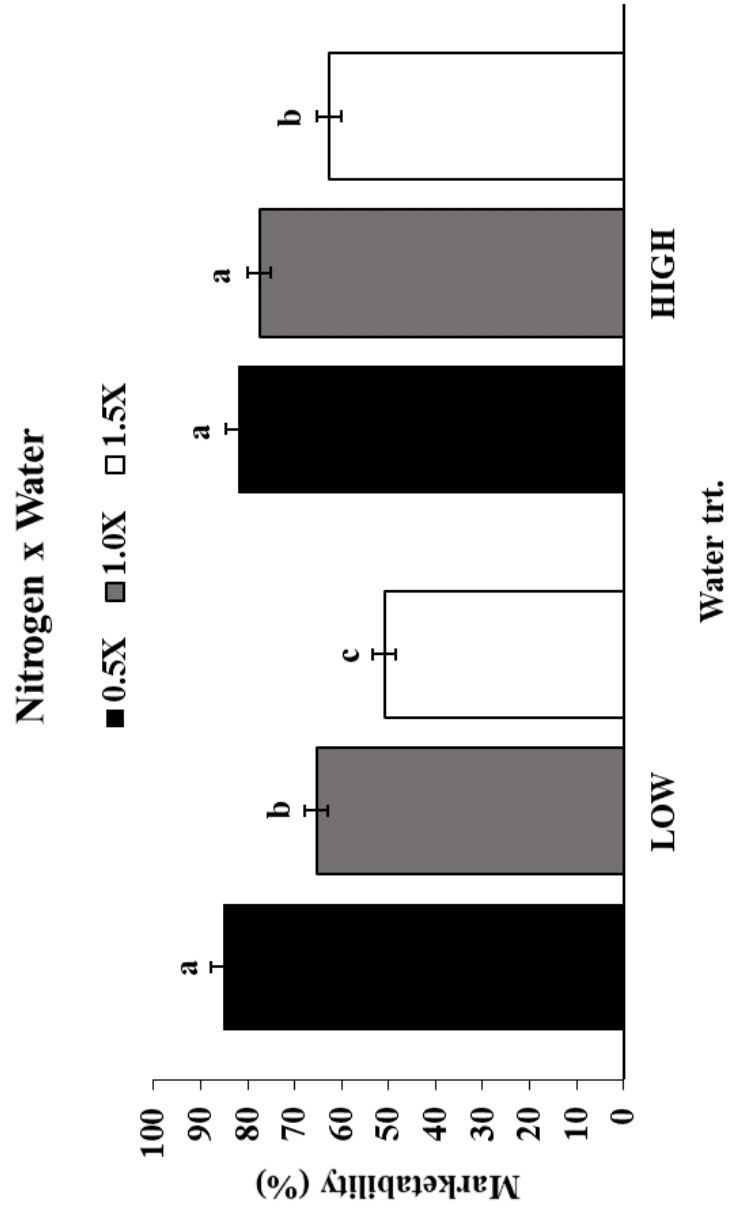


Figure 2.6

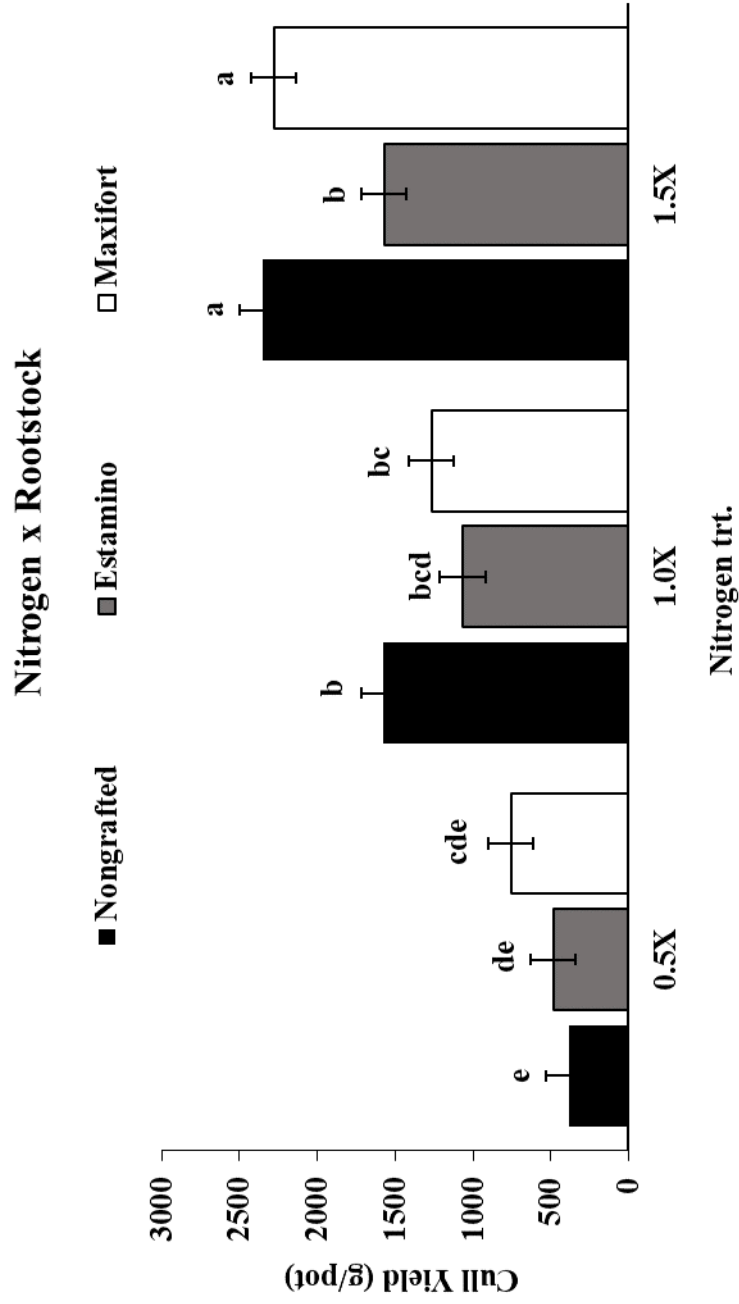


Table 2.2

Rootstock	Nitrogen	Water	Cull Fruit			Marketable Fruit			Total Fruit			Marketability (%)	
			Yield (g/pot)	No. of fruit	Avg fruit wt (g/fruit)	Yield (g/pot)	No. of fruit	Avg fruit wt (g/fruit)	Yield (g/pot)	No. of fruit	Avg fruit wt (g/fruit)	Yield	No. of fruit
Estamino	0.5X	Low	348.62	3.50	118.92	3653.70	25.17	146.33	4337.87	31.83	139.25	84.77	80.87
		High	619.28	5.00	113.52	4566.37	28.83	158.53	5375.55	35.67	150.49	85.31	80.97
	1.0X	Low	1263.17	13.67	89.20	3641.70	26.67	132.86	5183.80	43.17	118.44	68.07	61.13
Maxifort	1.5X	High	869.18	7.50	109.43	4747.26	33.19	143.19	5877.85	43.40	135.55	81.56	77.01
		Low	1434.75	18.83	80.25	2663.77	24.00	110.43	4116.22	43.17	96.50	63.31	55.88
	High	1707.03	17.67	95.78	4247.10	31.50	134.85	6056.83	50.83	117.88	69.64	60.87	
Nongrafted	0.5X	Low	773.37	8.17	98.65	4398.37	29.17	155.11	5588.18	41.83	134.19	78.78	69.71
		High	738.17	6.17	109.25	4039.15	27.83	146.11	5152.52	37.67	137.04	78.58	73.62
	1.0X	Low	1327.90	13.67	100.37	2817.25	21.50	128.62	4155.90	35.33	115.37	65.29	59.21
Standard error	1.5X	High	1206.25	11.17	109.74	4553.85	32.50	140.56	5919.81	45.50	130.22	77.41	72.17
		Low	1949.68	23.67	86.40	2040.35	17.67	113.34	4134.60	60.00	85.63	48.25	37.97
	High	2607.55	87.67	94.35	3343.47	24.67	136.00	5977.85	112.67	102.85	55.96	41.75	
Standard error	0.5X	Low	164.78	2.17	82.06	4116.43	28.67	144.27	4460.02	32.83	136.08	91.94	86.87
		High	605.78	6.50	100.30	4786.62	34.00	142.83	5763.80	44.67	128.98	82.33	75.01
	1.0X	Low	1889.38	19.00	99.33	3874.98	32.83	117.97	6113.88	56.17	108.66	62.70	58.12
Standard error	1.5X	High	1260.42	11.50	119.80	4946.17	34.67	142.44	6692.93	51.67	130.93	73.54	67.73
		Low	2598.60	34.83	78.71	2013.95	18.00	108.39	4675.70	53.67	88.26	41.16	33.45
	High	2102.85	24.50	85.12	3776.40	30.67	123.33	6026.12	57.17	105.10	62.22	53.34	
Standard error			205.57	14.90	11.70	466.69	3.44	5.64	521.68	15.77	7.38	4.39	5.33

Table 2.3

Type III Tests of Fixed Effects	Cull Fruit			Marketable Fruit			Total Fruit			Marketability (%)	
	Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit	Avg fruit weight	Yield	No. of fruit
Water	0.9694	0.5237	0.0354	<.0001	0.0001	0.0001	<.0001	0.2312	0.0001	0.0014	0.01
Rootstock	0.0063	0.3343	0.6485	0.4438	0.3036	0.1142	0.512	0.3303	0.1962	0.0157	0.0213
Nitrogen	<.0001	0.0027	0.0116	<.0001	0.0055	<.0001	0.0806	0.0211	<.0001	<.0001	<.0001
Water*Rootstock	0.2393	0.2959	0.8938	0.8023	0.9571	0.5334	0.942	0.6092	0.7266	0.9838	0.9799
Nitrogen*Water	0.0275	0.3907	0.7824	0.0659	0.2046	0.0060	0.1177	0.5102	0.0697	0.0045	0.0332
Nitrogen*Rootstock	0.0197	0.3931	0.3029	0.4603	0.2629	0.9600	0.1498	0.2571	0.9054	0.0559	0.1342
Nitrogen*Water*Rootstock	0.0982	0.2784	0.8426	0.5742	0.3819	0.2645	0.1617	0.6523	0.8057	0.2835	0.2355

Pr > F