Maximizing quality in grafted tomato production systems

by

Tricia Malynn Jenkins

B.S., Colorado State University, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
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Abstract

Vegetable grafting is a unique technology that can be easily adopted by growers to improve pest and disease resistance, provide abiotic stress tolerance, and increase marketable yields. The production of grafted vegetable transplants and their use in different production systems is increasing in North America. Tomatoes (*S. lycopersicum* L.) are currently the most popular grafted crop. The expansion of this technology relies on the availability of high-quality grafted tomato transplants as well as the ability of grafted plants to improve production and maintain or improve fruit quality for growers. The overall objectives of this dissertation were threefold: (i) to review the literature on tomato rootstock effects on tomato fruit quality (ii) to identify quality and performance impacts of grafted tomato transplants following abiotic stress from the supply chain (iii) investigate how rootstocks can influence the yield performance and fruit quality of a high-lycopene cultivar ('Tasti-Lee') grown in a high tunnel. The literature review found that changes in tomato fruit quality traits from rootstocks are wide-spread and highly subject to rootstock-scion and rootstock-scion-environment interactions. However, there are numerous reports that fruit from plants grafted to vigorous rootstocks have a larger average fruit size, lower soluble solid content (SSC), lower ascorbic acid (AsA) content, and higher titratable acidity (TA). Future investigations should focus on identifying the underlying mechanisms of fruit quality changes from grafting to tomato rootstocks. For the second objective, we found that exogenous ethylene exposure reduced chlorophyll fluorescence (F_v/F_m) and caused leaf epinasty of grafted seedlings. Yet, damaged plants recovered and had similar growth parameters to the control plants three weeks after transplanting. Non-ideal transportation conditions were also assessed by exposing plants to 35 °C for 6 to 48 hours during long-distance (72-hr) transportation. Similarly, the plants experienced physiological stress as measured by F_v/F_m, but all plants survived transplanting and early growth was not impacted. In both of these experiments, grafted plants were able to better maintain F_v/F_m and reduce the severity of symptoms such as epinasty and succulent elongation compared to nongrafted plants. The results from this objective indicate that transplant quality can be negatively affected from the stress conditions tested, but early growth was not inhibited. These results also suggest that grafted plants may be able to better tolerate abiotic stress at the seedling stage compared to nongrafted plants. In regards to the third objective, a three-year high tunnel trial was conducted at the Olathe

Horticulture Research and Extension Center to assess the yield and fruit quality impacts of five rootstocks grafted to the premium cultivar ‘Tasti-Lee’. Fruit quality was determined by SSC, TA, antioxidant capacity, AsA content, lycopene content, carotenoid composition, and fruit firmness. Grafting with ‘Maxifort’, ‘Fortamino’, ‘Estamino’, and ‘DRO-141-TX’ significantly increased marketable yields by 31.5%-47% above non-grafted plants. Conversely, the rootstock ‘RST-04-106-T’ did not provide any yield benefit. All of the rootstocks increased the average fruit weight by 12%. ‘RST-04-106-T’ was the only rootstock that altered fruit quality. This rootstock produced fruit with the highest SSC which was significantly higher than fruit from the rootstock ‘Maxifort.’ Moreover, ‘RST-04-106-T’ altered the relative composition of carotenoids compared to the nongrafted treatment by limiting β-carotene content in relation to the high lycopene concentrations. These results indicate that, with the proper rootstock selection, the cultivar ‘Tasti-Lee’ can be successfully integrated into high tunnel grafting systems without compromising its characteristic fruit quality attributes.

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Dedication

I want to dedicate this dissertation to my Uncle, John Oliver Bennett—a fellow scientist and a great role model. He did not get the opportunity to see me finish my degree, but he would have read every word of this dissertation with the utmost enthusiasm.

Chapter 1-Literature review: Evaluation of grafted tomato fruit quality under normal and abiotic stress conditions

Abstract

Grafting is an important tool for improving tomato performance and yields in a wide range of production systems worldwide. Given the large consumer demand for tomatoes and the recent interest in tomatoes as a functional food, it is important that fruit quality is also maintained in grafting production systems. Often, the fruit quality effects induced by grafting are dependent on scion-rootstock combinations and scion-rootstock-environment interactions. This makes identifying clear effects from commercial rootstocks difficult. This review summarizes the recent findings regarding the grafting effects on tomato quality (appearance, texture, organoleptic quality, and nutritive quality) under both normal and stressed conditions. The possible mechanisms for grafting-induced changes to fruit quality are suggested and reviewed from the literature. The most consistent fruit quality changes are often associated with the use of vigorous rootstocks and include increases in fruit size, reductions in fruit soluble solid content (SSC), increases in titratable acidity (TA), and reductions in ascorbic acid content.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated and consumed vegetables worldwide (Bertin and Génard, 2018). The Food and Agriculture Organization (FAO) estimates that over 180 million metric tons of tomatoes were produced globally in 2019 (FAOSTAT, 2019). In the United States alone, the utilized production value of fresh and processed tomatoes exceeded 1.6 billion dollars in 2019 (USDA NASS, 2020). Tomatoes are a significant source of nutrition for the world due to their vast consumption and nutritive quality (Dorais et al., 2008). Epidemiological and clinical studies have found associations between the consumption of tomatoes and reduced risk or markers of chronic diseases like cancer, cardiovascular disease, macular degeneration, and metabolic syndrome (Cheng et al., 2017; Cuevas-Ramos et al., 2013; Giovannucci, 2005; Tsitsimpikou et al., 2014).Tomatoes are a rich source of functional compounds such as vitamin C, provitamin A, carotenoids, phenolic compounds, and minerals in the human diet (Canene-Adams et al., 2005). The antioxidant function of many of these compounds are considered to be the reasons for the disease prevention and human health benefits from tomato consumption (Basu and Imran, 2007).

From both a producer and a consumer perspective, tomato fruit quality involves traits related to appearance, flavor and nutritive value. Increasingly, consumers are dissatisfied with the flavor of modern tomato cultivars (Klee and Tieman, 2013). Intensive breeding and the selection of agronomic traits have inadvertently diminished tomato flavor and nutrition, a phenomenon that has been proven in other intensively bred crops, such as wheat (Klee and Tieman, 2013). Also, many open-field produced tomatoes are harvested at the immature green stage and subjected to postharvest conditions that result in poor flavor (Baldwin et al., 2011). In recent years, there has been a greater focus on breeding for fruit flavor. Seed companies and

breeders now offer diverse selections of cultivars that vary in nutrition and flavor (Dorais et al., 2008). Additionally, manipulations of environmental conditions such as light, temperature, and irrigation regimes are investigated as methods to improve tomato quality (Bertin and andGénard, 2018; Gude et al., 2020).

Vegetable grafting is a technology used by growers worldwide to provide crop tolerance to economically devasting soilborne pathogens (Lee et al., 2010; Singh et al., 2017). Grafting tomatoes to selected rootstocks can also provide tolerance to abiotic stressors such as high and low temperatures, soil salinity, and water stress (Abdelmageed and Gruda, 2009; Fernández-Garcí et al., 2004; Rivero, Ruiz, Sánchez, et al., 2003; Suchoff et al., 2018). When no disease pressure is present, vigorous rootstocks can also improve yields, especially in greenhouse and high tunnel production (Lang et al., 2020; King et al., 2010; Lee and Chambers, 2010; Masterson et al. 2016; Meyer et al., 2021). Thus, rootstock breeding has historically been focused on the abovementioned traits (Kyriacou et al., 2017; Miao et al., 2019). Many commercial tomato rootstocks are interspecific hybrids of *S. lycopersicon* and *S. habrochaites*, which have high-vigor and multiple resistances to soilborne pathogens and viruses (Mahmoud et al. 2020). Increasingly, the impacts on fruit quality from grafting with commercial rootstocks in production systems without significant disease pressure are being investigated. In tomato, the fruit quality impacts attributed to grafting are varied and often contradictory. This is likely due to the diverse experimental climatic conditions, cultural practices, prodcution systems and grafting combinations assessed. Many of the fruit quality traits are also subject to scion-rootstock and scion-rootstock-environment interactions. Yet, grafting has also been investigated for the potenital to improve tomato quality (Flores et al., 2010). This review aims to systematically summarize recent findings concerning the effects of grafting on the physical, organoleptic, and

nutritional quality parameters of tomatoes. The focus of the review is on the use of grafted plants in non-stressed conditions, as well as those subjected to abiotic stress. The possible mechanisms for rootstock influence on fruit quality are also reviewed and suggestions for future research are considered.

Fruit Size, Color and Texture

Fruit Morphology. The average fruit weight of tomatoes is often increased due to grafting, with average increases ranging from 10% to 46% as compared to non-grafted controls (Djidonou et al., 2017; Koleška et al., 2018; Lang et al., 2016; Mahmoud, 2020; Mauro et al., 2020a; Moreno et al., 2019; Pogonyi et al., 2005; Rahmatian et al., 2014).. Despite the potential for increased tomato yields, an increase in fruit weight is an important quality consideration that can be positive or negative depending on the intended use and marketing of the tomato. An increase in average fruit weight is often associated with the use of vigorous interspecific rootstocks, such as 'Beaufort' (Casals et al., 2018; Djidonou et al., 2016, 2017; Á. Pogonyi et al., 2005; Turhan et al., 2011), 'Maxifort' (Djidonou et al., 2017; Koleška et al., 2018; Lang et al., 2020), 'Multifort' (Djidonou et al., 2016, 2017) and 'King Kong' (Rahmatian et al., 2014). Mauro et al. (2020a) found that the mean fruit weight increased by an average of 10% in all graft combinations when eight different cherry tomato scions were grafted to seven different interspecific hybrid rootstocks and grown in a greenhouse. In this same study, the use of an intraspecific hybrid as a rootstock did not significantly increase mean fruit weight.

The fruit expansion phase of tomato growth is regulated by the flow of water and assimilate into the fruit (Ho, 1996). It can be hypothesized that the increase in average tomato weight from grafting is related to the improved water and nutrient uptake by the vigorous rootstocks (Djidonou, Zhao, et al., 2013; Leonardi and Giuffrida, 2006). However, there have

also been reports of rootstocks having no impact on fruit weight when grafted to vigorous rootstocks (Qaryouti et al., 2007; Savvas et al., 2011; Schwarz et al., 2013), and two reports of grafting decreasing average fruit weight compared to non-grafted plants (Nicoletto et al., 2013b; Vinković Vrček et al., 2011). In the latter paper, there were significant reductions in fruit weight when the commercial cultivar 'Tamaris' was grafted to 'He-Man', 'Efialto', and 'Maxifort' rootstocks and grown in greenhouse conditions.

Impacts on fruit weight are often dependent on scion-rootstock combinations and environmental factors. For instance, the use of 'Beaufort' consistently increased fruit weight in the scions 'Yeni Talya', 'Swanson', and 'Beril'; but, the rootstock 'Arnold' only increased fruit weight of the scion 'Swanson' (Turhan et al., 2011). Lang et al. (2020) found that increases in fruit weight of the commercial hybrid 'BHN 589' was rootstock and year-dependent under high tunnel cultivation. In 2017, fruit weight increased in two out of the eight rootstocks assessed, while in the following year, increases in average fruit weight were found in five out of the eight assessed (Lang et al., 2020). Likewise, Casals et al. (2018) demonstrated that fruit weight increases incurred by grafting to the rootstock 'Beaufort' were scion and environment-dependent. Under conventional cultivation in high tunnels, average fruit weight was increased in 'Mando' and 'Montgri', but was significantly decreased in 'Egara.' However, under organic outdoor conditions, fruit size was only increased in 'Mando' (Casals et al., 2018).

A symptom of high salinity or water stress in tomatoes is reduced fruit weight (Koleška et al., 2018). Under normal salinity, 'Maxifort' was able to increase fruit weight in two different scions, and under high salinity, the grafted plants were able to prevent some of the fruit weight loss that was observed in nongrafted plants (Koleška et al., 2018). Yet, Savvas et al. (2011) found that grafting to three vigorous interspecific rootstocks did not increase average fruit weight

of ‘Belladona’ fruit under normal or elevated NaCl concentrations. However, the total yields were improved under elevated NaCl concentrations due to an increase in fruit per plant in the grafted treatments (Savvas et al., 2011).

There are only a few reports on the grafting effect on tomato fruit shape. Mauro et al. (2020b) found no differences in the shape index (ratio among longitudinal and transversal diameters) of ‘Sir Elyan’ grafted to three rootstocks withvarying degrees of vigor. ‘Sir Elyan’ is an oblong-shaped tomato, so any changes to the shape index would be an important quality consideration. Similarly, Mauro et al. (2020a) saw no difference in shape index of seven cherry tomatoes grafted to eight different rootstocks. Rather, the scion genotype more tightly controlled fruit shape (Mauro et al., 2020a). Qaryouti et al. (2006) also saw no difference in the fruit length or diameter of the commercial hybrid ‘Cecilia’ when grafted to ‘He-Man’ or ‘Spirit’. These findings are consistent with the general understanding that fruit shape is regulated by genotype (Bertin and Génard, 2018). Yet, the rootstock ‘Beaufort’ significantly increased the fruit index (diameter/length) in three commercial scions grown in a greenhouse (Turhan et al., 2011). Similarly, Casals et al. (2018) found that ‘Beufort’ increased the length and width of three scion cultivars when grown under conventional, high tunnel cultivation. Yet, grafting did not influence the fruit length and width of the same graft combinations under outdoor and organic cultivation. This indicates that this trait could be influenced by certain scion-rootstock combinations and environmental conditions.

Color. Color is another important fruit quality attribute that alters with development and ripening processes. Few studies have reported on the impact of grafting on objective color. Brajovic et al. (2012) found significant influences of rootstock on the a*/b* and L* index values on two different scions and fruit harvested at three different ripeness stages. Average a*/b* values were

lower and L* were higher when scions were grafted to ‘Body F1’ compared to those grafted to ‘Robusta F1’ or the nongrafted plants. However, the non-grafted scion cultivars were also statistically different in color parameters (Brajovic et al., 2012). The authors conclude that fruit from plants grafted to ‘Body F1’ were slower to ripen. Mauro et al. (2020a) observed that cherry tomato cultivars grafted to interspecific hybrid rootstocks, had fruit with significantly lower a* coordinates compared to those grafted to intraspecific hybrid rootstocks. Again, the authors found more variation in color due to scion genotype than scion-rootstock influence. Di Gioia et al. (2010) conducted a descriptive sensory analysis with a trained panel on grafted and nongrafted ‘Cuore di Bue’ fruit harvested at the pink stage. The panel found no significant differences in the intensity of red color between fruits.

Texture. Tomato fruit texture is an important quality trait that can be influenced by genotype, environmental growing conditions, and postharvest handling conditions (Aurnd et al., 2012). Fruit firmness is the second most important quality parameter that influences sensory perception and consumer acceptance (Causse et al., 2010; Causse et al., 2003). During ripening, the middle lamella dissolves, and enzymes begin breaking down cell walls, leading to decreased fruit firmness (Bertin and Génard, 2018). Grafting often does not impact fruit firmness (Barrett et al., 2012; Khah et al., 2006; Lang et al., 2020; Lang and Nair, 2019; Mauro et al., 2020a; Schwarz et al., 2013). Nonetheless, some rootstock and scion combinations have influenced this quality trait. Mauro et al. (2020b) found that rootstock choice influenced the firmness of ‘Sir Elyan’ tomatoes when harvested at two different ripeness stages: breaker and turning. ‘Sir Elyan’ tomatoes grafted to ‘He-Man’ were firmer than those grafted to ‘Interpro’ or ‘Armstrong.’ Mahmoud et al. (2020) identified that the interspecific rootstock *(S. lycopersicum var. cerasiforme x S. galapagenese*) consistently increased fruit firmness of greenhouse-grown ‘Santa Cruz Piedade’

scions over two years. Other reports suggest that grafting can lead to faster ripening of tomatoes (Brajovic et al., 2012; Leonardi and Giuffrida, 2006). Although fruit firmness was more influenced by scion cultivar, Brajovic et al. (2012) found that those grafted to 'Body F1' or 'Robusta F1' were significantly less firm when harvested at the red ripe stage compared to the nongrafted tomatoes. There were no significant differences in firmness due to grafting when fruit was harvested at the orange or light red stage (Brajovic et al., 2012).

Differences in fruit firmness among grafted and non-grated plants have also been observed under abiotic stress (Milenković et al., 2020). Under shade conditions, nongrafted fruits from 'Big Beef' and 'Optima' decreased in fruit firmness, but grafting to 'Maxifort' reduced the proportion of softened fruits (Milenković et al., 2020). Future investigation should focus on how ripening during storage may be altered from grafting because fruit firmness at harvest is not a reliable indicator of firmness losses during storage (Aurnd et al., 2012).

Organoleptic quality

Sugars. Sugar concentration is considered one of the most important flavor attributes of tomatoes and is linked to consumer acceptance (Causse et al., 2010). Sugars make up the majority of soluble solids in tomatoes (Bertin and Génard, 2018). There are several accounts of decreased soluble solid content (SSC) or reducing sugar concentrations of tomato fruit from grafted plants compared to fruit from nongrafted plants (Al-Harbi et al., 2017; Casals et al., 2020; Mauro et al., 2020a; Milenković et al., 2020; Moreno et al., 2019; Nicoletto et al., 2013; Pogonyi et al., 2015; Schwarz et al., 2013; Turhan et al., 2011). In the previously listed citations, the reported decreases in SSC as compared to the nongrafted treatments typically range from 9% to 13%. Some graft combinations may lead to more significant reductions in SSC, such as a 46% and 40% SSC reduction when 'Cecilia' was grafted to 'He-Man' and 'Spirit' (Qaryouti et al., 2006).

In other examples, grafting did not affect the SSC (Barrett et al., 2012; Di Gioia et al., 2010; Djidonou et al., 2016, 2017; Khah et al., 2006; Qaryouti et al., 2007; Savvas et al., 2017). Often, harvest period (Djidonou et al., 2017; Di Gioia et al., 2010), cultivation system (Qaryouti et al., 2006) or year-to-year variation (Lang et al., 2020; Barrett et al., 2012) contribute to greater sources of variation in the SSC of tomato fruit. Increased SSC in tomato fruit has also been attributed to grafting (Mahmoud, 2020; Rahmatian et al., 2014; Sánchez-Rodríguez et al., 2012).

This quality trait is highly dependent on scion-rootstock combination, and it is common for decreases in SSC to only occur in some of the combinations tested (Lang et al., 2020; Qaryouti et al., 2006). As an exception, Turhan et al. (2011) found significant reductions in all combinations of three scions (Yeni Talya', 'Swanson', 'Beril') and two rootstocks ('Beaufort' and 'Arnold') under soil cultivation in a greenhouse. However, the severity of total sugar (%) reductions depended on the scion-rootstock combination. Two studies show that reductions in total sugars from grafting to 'Briegor' and 'Maxifort' were more severe in the cherry tomato scion 'Piccolino' than in the larger-fruited 'Classy' (Krumbein and Schwarz, 2013; Schwarz et al., 2013). The total sugars of grafted 'Piccolino' were reduced under normal and low radiation (Krumein and Schwarz, 2013) and normal and low potassium supply (Schwarz et al., 2013) as compared to nongrafted plants. Similarly, Mauro et al. (2020a) found that rootstock selection and not a scion-rootstock interaction were responsible for the SSC reductions from seven different cherry tomato scions. Specifically, grafting to the interspecific hybrid rootstocks (S. lycopersicum x S. habrochaites and S. lycopersicum x S. peruvianum) reduced SSC content, while grafting to intraspecific hybrids and the interspecific (S. lycopersicum x S. pimpinellifolum) did not influence SSC.

Interestingly, Nicoletto et al. (2013) reported that only fructose concentrations were lowered due to grafting, whereas glucose was the same among grafted and nongrafted 'Profitto' beefsteak tomatoes. This could be important in relation the sweetness of the tomatoes because fructose is perceived as sweeter than glucose. Krumbein and Schwarz (2013) and Moreno et al. (2019) found reductions in both glucose and fructose of grafted tomatoes.

There are a few different mechanisms that could decrease SSC in fruit of grafted plants. Vigorous rootstocks that enhance root and vegetative biomass could act as additional sinks and reduce photosynthate flow to the fruit (Martínez-Ballesta et al., 2010; Mauro et al., 2020a; Mauro et al., 2020b). This hypothesis is supported by Maruo et al. (2020a) who found that grafting to the most vigorous rootstock classes resulted in the most significant decreases of SSC in cherry tomatoes.

An increase in fruit load from rootstocks could also decrease the partitioning of assimilates to individual fruits (Klee and Tieman, 2013; Pogonyi et al., 2005). Fruit load can significantly alter fruit composition, fruit metabolism and influence gene expression during fruit development (Kromdijk et al., 2013). Additionally, higher fruit loads can result in slower fruit ripening, leading to a discrepancy in sugar content between grafted and nongrafted tomato fruit if they are harvested simultaneously (Kromdijk et al., 2013). Prudent et al. (2010) found that altered source-sink relationships due to fruit thinning increased the expression of two invertases (T1V1 and *β*-fructosidase), which likely explained the increases in SSC observed in thinned fruit. Asins (2015) identified two SSC QTLs (quantitative trait locus) that were mediated by rootstocks from a population of RILs (recombinant inbred lines) from a hybrid of *Solanum pimpinellifolium and S. lycopersicum* var. *Cerasiforme* and grown under moderate salinity. There was a significant negative correlation between SSC and total fresh weight harvested, which could

be explained by linked QTLs for these two scion traits. A co-location of fruit size and SSC traits was also identified in F2 populations of *S. pimpinellifolium* and *S. lycopersicum* (Hernández-Bautista et al., 2015).

Improved water uptake by vigorous rootstocks may increase the water content of the fruit, leading to a dilution of solutes (Mauro et al., 2020a; Milenković et al., 2020; E. Turhan et al., 2011). There are a few reports of vigorous rootstocks reducing fruit dry matter accumulation while maintaining or increasing average fruit weight (Krumbein and Schwarz, 2013a; Mauro et al., 2020a; Turhan et al., 2011). In these cases, the fruit also had reduced SSC, indicating a dilution effect. In peaches, an increase in SSC has been attributed to the rootstock influence on fruit size and not the rootstock itself (Shahkoomahally et al., 2021). Yet there is still evidence that SSC can decrease in grafted tomatoes without significant changes to fruit size (Gajc-Wolska et al., 2014; Qaryouti et al., 2007; Schwarz et al., 2013).

Transcriptomic studies performed in grafted *Cucurbitaceae* species have identified altered transcriptional processes involved in sugar metabolic pathways and fruit ripening processes in grafted plants. Aslam et al. (2020) identified seven differently expressed genes (DEGs) related to sugar metabolism and sugar transport in nongrafted watermelon and watermelon grafted to bottle gourd rootstock. The authors contribute the decrease in sugar content of grafted watermelons to these DEGs, which occurred at different stages of fruit development. Guo et al. (2021) also identified that genes related to phytohormone regulation, hormone transport, carbohydrate metabolic process, and other fruit quality and sweetness genes were downregulated in grafted watermelon at a late stage in fruit development. This altered gene expression, most notably in ABA signal regulators, acted to delay fruit ripening in grafted plants. The grafted watermelons were less sweet than the nongrafted watermelons on the same day after

pollination; however, they exceeded the nongrafted watermelons in sweetness, size and color after they were fully ripe (Guo et al., 2021). This points to the importance of sampling methods when comparing fruit quality traits that can vary widely through the ripening process. Transcriptome analysis of grafted and nongrafted tomatoes during fruit maturation and ripening would allow for further understanding of rootstock mechanisms in altering sugar content.

Acids. The acid content of tomatoes (predominately citric and malic acid) is also important for flavor quality. The accumulation of acids is regulated by fruit metabolic rate and vacuolar storage, which can be under genetic and environmental control (Etienne et al., 2013). Unlike SSC, acid content—commonly measured as titratable acidity (TA)—tends to increase in fruit from grafted plants (Khah et al., 2006; Lang et al., 2020.; Mauro et al., 2020a; Mauro et al., 2020b; Meyer et al., 2021; Sánchez-Rodríguez et al., 2012; Schwarz et al., 2013b; Turhan et al., 2011). Occasionally, fruit from grafted plants have had lower TA. For example, grafting to 'Profitto' to the rootstock 'Beaufort', decreased TA only from 4.30 to 4.39 (% citric acid) compared to nongrafted tomatoes (Nicoletto et al., 2013a). Milenković et al. (2020) also found that grafting two different cultivars to 'Maxifort' reduced the citric acid concentration but not the total acid content. There are also a number of examples of TA not being influenced by grafting (Al-Harbi et al., 2017; Barrett et al., 2012a; Di Gioia et al., 2010; Djidonou et al., 2016, 2017; Helyes et al., 2009; Savvas et al., 2011).

In some cases, the increase in TA is rootstock-dependent when using the same scion (Khah et al., 2006; Lang et al., 2020; Mauro et al., 2020b; Meyer et al., 2021). For instance, in a high tunnel system, the rootstock 'Maxifort' significantly increased fruit TA in the cultivar 'BHN 589', while the rootstocks 'RST-04-106-T' and 'RT 1028' did not affect fruit TA. Yet,

Schwarz et al. (2013) found increases in fruit TA in two different scions grafted to two different rootstocks grown hydroponically with increases ranging from 5.8-14.7% above the fruit from nongrafted plants. Mauro et al. (2020a) found a significant scion-rootstock interaction effect regarding TA content. The small-fruited cherry tomato scion classes observed in this study had a more notable TA increase caused by grafting (Maruo et al. 2020a). Krumbein and Schwarz (2013) also found that TA increased in the cherry tomato 'Piccolino' when grafted to the vigorous rootstocks 'Maxifort' and 'Brigeor', but the round truss cultivar 'Classy' did not significantly differ from the nongrafted tomatoes.

It appears that the acid content of grafted fruit increases without an increase in fruit dry matter (Krumbein and Schwarz, 2013; Mauro et al., 2020a; Schwarz et al., 2013; Turhan et al., 2011). Thus, a mechanism other than an increased flow of assimilates into the fruit must be acting to increase acid content (Schwarz et al., 2013).

Rootstocks may influence acid content through direct mechanisms of altered expression of genes involved in acid metabolism. Aslam et al. (2020) identified that the downregulation of 2-oxoglutarate dehydrogenase (OGDH) in citrate metabolism was involved with reduced citrate content of mature grafted watermelon. Rootstocks may also influence acid content through mechanisms such as altered mineral uptake, increased fruit load, or fruit shading from increased vegetative growth (Famiani et al., 2015). For instance, malic acid content of Shiraz and Chardonnay grape skin was higher when grafted to rootstocks that limit Cl uptake due to a negative relationship between malate and Cl (Gong et al., 2010). Indeed, select tomato rootstocks can limit Cl accumulation in leaves (Al-Harbi et al., 2017; Estañ et al., 2005; Wahb-Allah, 2014). Fruit load has also been positively correlated with tomato acid content (Bertin et al., 2000; Fanasca et al., 2007), which could explain increases in grafted plants. This trait is also highly

subject to environmental variation—such as temperature differences during fruit ripening (Bertin et al., 2000).

Sugar/Acid Ratio. The ratio between sugar and acid is important to the overall likeness of fresh-eating tomatoes as both the sugar and acid contribute to the sweetness and tomato aroma intensity (Causse et al., 2010). This ratio is often altered in grafted tomatoes due to either an increase in TA, a decrease of SSC, or a combination of both. Mauro et al. (2020b) attributed higher SSC/TA to increased SSC in the 'Sir Elyan' fruits grafted to the lowest-vigor rootstock. While, Mauro et al., (2020a) attributed a reduced SSC/TA to increases in TA of grafted small-fruited scion. Pogonyi et al. (2005) reported a 20% reduction in carbohydrate/acid ratio caused by a significant loss in SSC due to grafting despite comparable TA. However, Flores et al. (2010) identified a non-commercial rootstock from an RIL line developed from *S. lycopersicum* and *S. cheesmaniae* that significantly enhanced SSC and TA under normal and saline conditions for greenhouse grown 'Boludo' tomatoes. This points to the importance of rootstock selection and the possibility for developing rootstocks for tomato flavor enhancement under certain stress conditions (Flores et al., 2010).

Aroma Volatiles. Aroma volatiles in tomatoes contribute to the characteristic aroma and flavor attributes and improve the perception of sweetness (Bertin and Génard, 2018; Jukić Špika et al., 2021). Rootstocks have erratic influences on different aroma volatiles (Jukić Špika et al., 2021; Krumbein and Schwarz, 2013; Leonardi and Giuffrida, 2006; Mauro et al., 2020b). Krumbein and Schwarz (2013) found significant rootstock effects on the volatiles investigated. Carotenoid-derived volatiles were decreased in one scion-rootstock ('Classy'/'Brigeor') combination, while

lignin-related volatiles were enhanced due to grafting in both scions assessed (‘Classy and ‘Piccolino’). Mauro et al. (2020b) found that different aroma profiles of ‘Sir Eylan’ tomatoes grafted to three different rootstocks, with each rootstock increasing different volatiles. . Jukić Špika et al. (2021) noted a wide range of influences on aroma volatiles based on rootstock and scion-rootstock interactions. This study was able to identify a key few combinations and rootstocks that maximized aroma volatiles (Jukić Špika et al., 2021). It is unclear what is contributing to the differences in synthesis of volatiles in grafted tomatoes. Jukić Špika et al. (2021) suggest it could be due to an upregulation of transcripts of aroma-flavored genes, which has been demonstrated in grafted cucumber (Zhao et al., 2018).

Sensory Analysis. There have only been a few quantitative sensory analyses with trained panels performed on grafted tomato fruit (Mauro et al., 2020b, Cascals 2020; Di Gioia; Jukić Špika et al., 2021). Di Gioia et al. (2010) did not find any significant differences in sensory attributes between grafted and nongrafted ‘Cuore di Bue’ tomatoes. However, no differences were found in the organoleptic measurements of SSC or TA in this study either. ‘Sir Eylan’ tomato samples from three different rootstocks differed in 11 out of 16 flavor, odor, and texture attributes (Mauro et al., 2020b). Each rootstock increased the intensities of different attributes. Casals et al. (2018) found that grafting three different scions to ‘Beaufort’ decreased the intensities of sweetness, acidity, and taste intensity in organic outdoor production and conventional high tunnel production. However, one graft combination did not alter in “sweetness” and “taste intensity” in conventional production, revealing a significant grafting x environment interaction. This was mirrored by the results of the fruit SSC found in this study, which indicated that the impact of the rootstock ‘Beaufort’ was different for different scions and under different growing conditions.

The texture attribute of "skin permeability" was also decreased due to grafting in the conventional system, but not in the organic system (Casals et al., 2018).

Jukić Špika et al. (2021) also found that many sensory attributes were influenced by rootstock and scion-rootstock combinations. Yet, scion genotype revealed more significant effects on fruit appearance, odor, mouthfeel, and taste attributes. Differences in sensory attributes were also found from one year to the next. Yet, rootstocks caused changes in fruit sweetness in both years and one rootstock significantly enhanced fruit sweetness and resulted in the best overall quality score when grafted to one of the scions (Jukić Špika et al., 2021). Additionally, one rootstock increased flesh mealiness and flesh firmness. In general, grafting was not found to negatively affect the overall quality scores among the two scions assessed. The authors suggest that the varied sensorial and aroma attributes as influenced by rootstocks and scion-rootstock interactions can be utilized to grow tomatoes with targeted characteristics (Jukić Špika et al., 2021).

Functional compounds

*Vitamin C***.** Ascorbic acid (AsA) is a health-promoting antioxidant found in high amounts in tomatoe fruit. There are several reports of rootstocks reducing AsA content of tomatoes (Djidonou et al., 2017; Ilić et al., 2020; Koleška et al., 2018; Mauro et al., 2020a; Mauro et al., 2020b; Nicoletto et al., 2013a; Qaryouti et al., 2007; A. Turhan et al., 2011; Vinković Vrček et al., 2011; Wahb-Allah, 2014). These reductions are significant from a nutritional standpoint because reductions can often be large, ranging from 22% (Djidonou et al., 2016) to 47% (Qaryouti et al., 2007).

There are a few instances of grafting increasing AsA content in fruit (Fernández-Garcí et al., 2004; Rahmatian et al., 2014;). However, increases were low (3.5%) when 'Synda' was

grafted to ‘King Kong’ and grown hydroponically. Fernández-Garcí et al. (2004) found increases AsA content of ‘Fanny’ and ‘Goldmar’ grafted to the hybrid ‘AR-9704’ and grown with normal saline conditions, but concentrations were comparable under elevated salinity. Sánchez-Rodríguez et al. (2012) also found that AsA did not alter due to grafting under normal and high saline conditions. Others have also reported no effect on AsA from grafting under normal growing conditions (Barrett et al., 2012a; Savvas et al., 2017; Moreno et al., 2019).

In some cases, AsA content was dependent on the cultivation system as well as scion-rootstock combination. In soilless cultivation, ‘He-Man’ and ‘Spirit’ rootstocks significantly reduced AsA content of ‘Cecilia’ tomatoes by 39 and 47% (Qaryouti et al., 2007). Yet, when the plants were grown in soil, fruit from the nongrafted plants had significantly lower AsA concententrations comparedto those grown in soilless media, and grafting to ‘Spirit’ significantly increased AsA content (Qaryouti et al., 2007). The extent of decrease in AsA content is often dependent on scion-rootstock combination. ‘Maxifort’ acted to reduce AsA by 21.2%-29.3%, depending on the slicer-type tomato scion used (Ilić et al., 2020). ‘Cuore di Bue’ tomatoes decreased in AsA by 16% when they were grafted to ‘Beaufort’ and by 20% when grafted to ‘Maxifort’ (Di Gioia et al., 2010).

Mauro et al. (2020a) concluded that AsA content was influenced by cherry tomato scion, but significant scion-rootstock effects were found, and all grafted plants acted to lower concentrations. Rootstocks of the highest vigor resulted in the greatest decrease (-45%), yet concentrations were still lower for those grafted to low-vigor rootstocks as compared the nongrafted plants (Mauro et al., 2020a). Similarly, Mauro et al. (2020b) found that ‘Sir Eylan’ tomatoes grafted to the most vigorous rootstock acted to decrease AsA concentrations by 23% compared to concentrations obtained from the low-vigor rootstocks. Although the exact

mechanisms for how grafting works to alter AsA content is unknown, the above results support the commonly cited hypothesis that AsA is being redistributed or accumulated in other plant parts due to the added vigor caused by rootstocks (Di Gioia et al., 2010; Mauro et al., 2020a). As previously hypothesized as a mechanism for decreased SSC, AsA content may be diluted by grafted plants by an increase in fruit load or fruit water content.

Carotenoids. The majority of tomato carotenoids consist of lycopene, while beta-carotene, lutein, and other minor carotenoids are found in smaller amounts. Lycopene is a health-promoting compound due to its strong antioxidant capacity. Tomatoes are one of the most important sources of this functional compound in the human diet (Story et al., 2010). Grafting does not seem to have any consistent impacts on tomato carotenoid content. Rootstocks have increased lycopene (Brajovic et al., 2012; Fernández-Garcí et al., 2004; Moreno et al., 2019; Riga et al., 2016; Schwarz et al., 2013b), decreased lycopene (Gajc-Wolska et al., 2014; Helyes et al., 2009; Ilić et al., 2020; Koleška et al., 2018; Krumbein and Schwarz, 2013b; Nicoletto et al., 2013a; Qaryouti et al., 2007), and have had no effect on this attribute (Djidonou et al., 2016., 2017; Khah et al., 2006; Lang and Nair, 2019; Qaryouti et al., 2007; E Sánchez-Rodríguez et al., 2012; A. Turhan et al., 2011; Vinković Vrček et al., 2011). These contradicting results indicate that this trait is highly variable according to scion-rootstock combination. It is also important to note that differences found due to grafting are often not more significant than differences commonly observed between scion genotypes (Brajovic et al., 2012; Helyes et al., 2009). Nonetheless, it seems as though the papers reporting increases in carotenoids due to rootstocks are in the minority, and the most commonly used vigorous rootstocks such as ‘Beaufort,’ ‘Maxifort,’ and ‘Multifort’ typically decrease (Gajc-Wolska et al., 2014; Helyes et al., 2009; Koleška et al., 2018; Schwarz et al., 2013) or do not affect fruit carotenoid concentrations (Djidonou et al.,

2017; Djidonou, Gao, et al., 2013; A. Turhan et al., 2011; Vrcek et al., 2011). A few instances have found that certain scion-rootstock combinations could improve lycopene and β-carotene content under abiotic stress, such as water stress (Sánchez-Rodríguez et al., 2012) or low soil potassium (Schwarz et al., 2013). Yet, Koleška et al. (2018) found no improvement in lycopene content of two scions grown under salt stress by grafting to 'Maxifort.'

Carotenoid content is significantly altered during tomato fruit ripening. During ripening, lycopene accumulates in high concentrations, while other carotenoids such lutein and β-carotene are downregulated (Chattopadhyay et al., 2021). This process is responsible for the tomato red pigment and is regulated by many genes and metabolic pathways. It is still unclear how grafting may influence the ripening processes and consequently carotenoid concentrations during ripening and fruit storage. There are only very few studies that have investigated carotenoids at multiple ripeness stages or during shelf-life. In the majority of studies observing grafting effects on fruit quality, lycopene is measured at one time-point when the fruit has reached "red ripeness" either on the vine (Lang and Nair, 2019; E Sánchez-Rodríguez et al., 2012; Schwarz et al., 2013) or off of the vine (Djidonou et al., 2016, 2017). Sampling procedures could be causing the differences in the rootstock effect on carotenoid concentrations, especially if grafted and nongrafted plants have altered ripening patterns. Moreover, total carotenoids can differ between sampling dates and truss location (Coyago-Cruz et al., 2019). However, there is evidence that carotenoid accumulation during ripening is altered by scion-rootstock combination.

Brajovic et al. (2012) observed carotenoid concentrations (lycopene, β-carotene, and lutein) of fruit harvested at three different maturities (orange, light red). Grafted and nongrafted 'Amati' and 'Gardel' scions were evaluated, and the rootstocks used were 'Body' and 'Rubusta' (Brajovic et al., 2012). There was a significant impact due to grafting on the contents of the three

carotenoids, but the effect was different for all three maturity stages. For ‘Amati’, grafting had a significant effect on the ratio between lycopene and β-carotene due to an increase in relative lycopene above nongrafted plants at the orange and red stage. The rootstock ‘Body’ reduced lutein at all three maturity stages (Brajovic et al., 2012). Mauro, Rizzo et al. (2020) found rootstock differences in ‘Sir Eylan’ tomatoes at two different ripe stages (breaker and turning). The rootstock ‘He-Man’ had higher concentrations of lycopene and β-carotene at both stages, but when passing from breaker to turning, grafting to ‘Armstrong’ lead to a higher relative increase in β-carotene. Lastly, Ilić et al. (2020) observed one scion-rootstock combination (‘Big Beef’ and ‘Maxifort’) that had significantly lower lycopene content than the nongrafted tomatoes when measured on the day of harvesting. However, in this graft combination, lycopene increased during 15-day storage, while a different scion grafted to ‘Maxifort’ and nongrafted plants all decreased in lycopene concentration by day 15 as compared to their day 0. These results indicate that ripening processes and carotenogenesis is influenced by scion-rootstock combination.

Phenolics and total antioxidants. Phenolic compounds are a class of antioxidants found in tomatoes. Individual phenolic acids are rarely assessed in grafted tomatoes. Nicoletto et al. (2013a) found different patterns of chlorogenic acid concentrations during ripening between fruit from nongrafted ‘Profitto’ and ’Profitto’/’Big Power.’ In nongrafted tomatoes, chlorogenic acid increased from green to orang/red, followed by a decrease until the red ripe stage. However, when grafted to ‘Big Power’ chlorogenic acid increased until the red stage, indicating a rootstock influence on the biosynthesis of this compound (Nicoletto et al., 2013). Under drought stress, the use of a drought-tolerant rootstock increased concentrations of anthocyanins, flavonols, and phenols (Sánchez-Rodríguez et al., 2012). In general, total phenolics have a tendency to decrease

or be unimpacted under certain rootstock scion combinations (Djidonou et al., 2016; Ilić et al., 2020; Koleška et al., 2018; Nicoletto et al., 2013b; Riga et al., 2016; Vrcek et al., 2011).

Total antioxidants assays such as DPPH, FRAP, ORAC, and TEAC are also measured to assess grafted tomato nutritional quality. Similar to the phenolics, total antioxidants have been shown to decrease due to grafting (Djidonou et al., 2016; Koleška et al., 2018; Mauro et al., 2020b; Nicoletto et al., 2013a; Qaryouti et al., 2007; Riga et al., 2016). However, these effects are also subject to scion-rootstock interactions. Mauro et al. (2020b) found that the rootstock 'He-Man' increased total antioxidants (DPPH) in 'Sir Eylan' tomatoes compared to two other rootstocks, which mirror the increased AsA and carotenoid content found in this graft combination. Djidonou et al. (2016) noted that grafting decreased the lipophilic fraction of the ORAC assay, but the hydrophilic fraction was comparable to the nongrafted tomatoes.

The reduced antioxidant concentrations in grafted plants could be related to the rootstock tolerance to stress. Phenolics compounds and other antioxidants are important in maintaining oxidative balance in plants (Ahmad et al., 2010). Certain tomato cultivars increase antioxidant concentrations as a response to drought stress (Klunklin and Savage, 2017; Sánchez-Rodríguez et al., 2010) or heat stress (Rivero, Ruiz, and Romero, 2003). Rivero et al. (2003) found that when the heat-resistant cultivar 'RX-335' was used as a rootstock, the plants performed better under heat stress conditions for 30-days than nongrafted plants. But, the grafted plants produced significantly lower total phenols and *o*-diphenols in the leaves than nongrafted plants, indicating the rootstock had other mechanisms for resisting thermal stress (Rivero et al., 2003).

Fruit mineral content. Tomatoes provide a source of key trace elementsand minerals in the human diet (Fernández-Ruiz et al., 2011). Increases in mineral concentrations and trace elements in shoots and roots have been associated with vigorous rootstocks such as 'Maxifort' (Kumar et

al., 2015), ‘Unifort’ (Kumar et al., 2015; Al-Harbi, 2016), and ‘Beaufort’ (Leonardi and Giuffrida, 2006). Only select scion-rootstock combinations have increased mineral concentrations in fruit. The rootstock ‘He-Man’ increased fruit Ca (ppm) of ‘Big Power’ when grown in a greenhouse, but not in open field conditions (Khah et al., 2006). The drought-tolerant rootstock ‘Zarina’ significantly increased macronutrient concentrations (Ca K, Mg) in the fruit of water-stressed plants but did not increase trace elements Zn, Mn, Fe, and Cu (Sánchez-Rodríguez et al., 2012). Asins et al. (2020) identified 21 rootstock-mediated QTLs for fruit mineral concentrations, including Al, Ca, Mg, Mn, Na, P, S, Si, and Fe. Grafting ‘Boludo’ on a population of RILs from a hyrbrid of *S. pimpinellifolium and S. lycopersicum* var. *Carasiforme* increased Fe uptake in low-iron conditions and enhanced the overall harvestable Fe in fruit in 89% of the rootstocks (Asins et al., 2020).

Blossom-end rot is an important physiological disorder that limits marketability in tomato fruit and is associated with Ca deficiency in early fruit expansion (Ho and White, 2005). Rootstocks or scion-rootstock combinations that increase uptake and movement of Ca and/or K may alleviate the incidence of BER. ‘Piccolino’ and ‘Classy’ scions had a lower incidence of BER when grafted to ‘Brigeor’ and ‘Maxifort’ under normal and low K conditions. BER was also reduced under low light and normal light conditions for ‘Piccolino’, but only under normal light for ‘Classy’ when grafted to the same rootstocks, revealing a R x S x E interaction. Lang et al. (2020) found a large season to season variation in the rootstock effect of BER e.g., the rootstock ‘RST-04-106’ significantly increased incidence in one year and reduced incidence in the next. While, ‘Esatmino’ and ‘Maxifort’ reduced BER occurrence in both years compared to nongrafted and self-grafted plants (Lang et al., 2020). Asins et al. (2020) and Asins et al. (2015)

identified rootstocks in the RIL populations that had no incidence of BER. Therefore, the trait could be selected to provide resistance to BER in grafted plants.

Heavy metal accumulation in fruit is a significant food safety concern. Cultivating horticultural crops with treated wastewater or in heavily contaminated soils can result in the accumulation of arsenic, cadmium, lead, and mercury in edible portions (Edelstein and Ben-Hur, 2018). Tomato consumption is not likely to exceed daily intake limits heavy metals such as Cd and Pb (Luis et al., 2012), even when grown in soil with moderate heavy metal contamination (Angelova et al., 2009). Yet, Asins et al. (2020) reported that 26% of the rootstock RIL population evaluated accumulated between 27 and 338 ppm of Al in the fruit, which is not typical for tomatoes and poses a food safety concern. Additionally, Al content in the fruit was correlated with unmarketable, deformed fruit (Asins et al., 2020). Tomato rootstocks may also be able limit uptake and translocation of heavy metals, such as cadmium (Kumar et al., 2015) and arsenic (Stazi et al., 2016), which can be useful in maintaining crop performance and fruit quality in soils with heavy metal contamination.

Conclusion

Grafting can have wide-ranging impacts on tomato fruit quality. Which fruit quality trait is altered and to what degree is often dependent on the selected scion for any given rootstock. The average fruit size is often increased due to grafting and is associated with the use of vigorous, interspecific rootstocks. A reduction in sugar or SSC is also common in grafted tomatoes, and this trait is subject to scion-rootstock and scion-rootstock-environment interactions. The reduction in sugars due to grafting is typically not more drastic than variations that can be attributed to other pre-harvest factors such as genotype, cultural practices, irrigation regimes, or harvest date. Several studies have also demonstrated a grafting-induced increase in

TA. These alterations to fruit sugars and acids could decrease the sugar/acid ratio in grafted fruit and lead to reduced sensorial quality.

In terms of nutritional value, there seems to be a clear trend between the use of tomato rootstocks and reduced AsA content in tomato fruit. Vigorous rootstocks cause the most considerable reductions in AsA content. Grafting can influence and often reduce the concentrations of carotenoids, phenolic compounds, and total antioxidants, but this effect is also highly subject to scion-rootstock combination. Additionally, there is evidence that grafting can influence the ripening patterns of tomatoes. Thus, the simultaneous harvesting of grafted and nongrafted fruit can result in measured differences in primary and secondary metabolite concentrations—which could be due to varying maturity stages and not from a direct rootstock effect. Other incidental rootstock effects such as altered sink-source relationships and increased fruit loads are possible mechanisms influencing fruit quality traits. Future transcriptomics work in grafted tomatoes should identify possible mechanisms for fruit quality impacts.

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Chapter 2-Evaluating ethylene sensitivity and exogenous ethylene impact on quality and early growth of grafted and nongrafted tomato seedlings

Abstract

Ethylene sensitivity of grafted and nongrafted tomato seedlings was examined. The concentration of exogenous ethylene that produced symptoms was between 1 $\mu L \cdot L^{-1}$ and 10 $\mu L \cdot L^{-1}$ regardless of graft status. Symptoms of ethylene exposure included leaf epinasty, decreased F_v/F_m, and increased plant height (nongrafted only). Grafted plants maintained higher F_v/F_m than nongrafted plants in response to higher ethylene concentrations. Ethylene-damaged plants showed comparable growth to the control plants three weeks after transplanting, but transplant quality was diminished due to ethylene exposure at high concentrations.

Introduction

Grafted plants can provide resistance to soilborne diseases and abiotic stress, enhance plant vigor, and increase yields (Louws, Rivard and Kubota, 2010; Schwarz *et al.*, 2010). The production of grafted tomato (*Solanum lycopersicum* L.) plants has been increasing in North America (Ertle and Kubota, 2019). An estimated 36 million grafted tomato plants are produced annually by a relatively few numbers of high-volume grafting nurseries in Canada, Mexico, and the USA, and most are shipped long distances to reach growers (Ertle and Kubota, 2019). Grafted plants have greater value than nongrafted vegetable transplants; therefore, transplant quality needs to be maintained during the supply chain. One potential source of quality deterioration in commercial greenhouses, storage facilities, and during shipping is exogenous and endogenous ethylene accumulation.

Tomatoes are classified as highly sensitive to ethylene (Edelman and Jones, 2014.). During greenhouse production, plants may be exposed to long periods of low concentrations of ethylene—in the range of 0.025 $\mu L \cdot L^{-1}$ to 0.2 $\mu L \cdot L^{-1}$ (Jones and Edelman, 2013). The source of exogenous ethylene is often from malfunctioning greenhouse heaters (Jones and Edelman, 2013). Higher concentrations of ethylene—around 1 $\mu L \cdot L^{-1}$—can result in plant symptoms such as leaf epinasty and leaf, bud, and flower abscission in tomatoes (Edelman and Jones, 2014). Exposure to such higher ethylene concentrations is more typical during the postproduction of bedding plants, i.e., during packaging, shipping, and retailing (Jones and Edelman, 2013). For instance, ethylene accumulation during long-distance shipping can cause flower abortion and abnormal truss development of mature grafted tomato transplants (Kubota and Kroggel, 2011). It is unclear how ethylene exposure during production or postproduction supply chain impacts vegetative grafted tomato transplants without visible flowers. The objectives of this study were to 1.) determine the ethylene sensitivity of vegetative grafted and nongrafted tomato seedlings and 2.) determine if a prolonged (4-d) ethylene exposure will impact transplant quality and early growth after transplanting.

Materials and Methods

Plant materials

Grafted and nongrafted tomato seedlings were grown in a greenhouse at the Kansas State University Olathe Horticulture Center (38.884347, -94.993426). ‘Cherokee Purple’ (Johnnys Selected Seeds; Winslow, ME) was selected for the scion, and ‘Maxifort’ was selected for the rootstock (DeRuiter Seeds; St. Louis, MO). The seedlings were splice/tube grafted and healed, according to Rivard and Louws (2011). The healing chambers were located in the greenhouse and covered with polyethylene film and 55% shade cloth. The plants remained in the healing

chambers for 10 d. After healing, the plants were returned to the greenhouse and grown for one week before beginning the experiment. Nongrafted 'Cherokee Purple' seeds were sown two weeks after the grafted plants. Both grafted and nongrafted plants had three to four true leaves at the start of the experiment. The plants were transferred to the Postharvest Physiology Lab at Kansas State University Olathe for the ethylene exposure treatments.

Experiment 1: Ethylene treatment

Exogenous ethylene treatment. The grafted and nongrafted plants were randomly placed in four separate plexiglass chambers, with 25 grafted and 25 nongrafted plants in each chamber. The chambers were subjected to one of the following exogenous ethylene treatment concentrations: 0 $\mu L \cdot L^{-1}$(control), 0.1 $\mu L \cdot L^{-1}$, 1 $\mu L \cdot L^{-1}$, and 10 $\mu L \cdot L^{-1}$. The internal temperature in the chambers was 18.7 °C ± 0.3, and the relative humidity ranged from 96% to 99%. The chambers remained on the laboratory bench and were exposed to a natural photoperiod near a window. The ethylene concentrations were confirmed with a gas chromatographer with a flame ionization detector and a 6' hayesep D column (SRI Instruments; Torrance, CA).

Symptom evaluation All measurements were made at day 0 immediately before the start of the experiment and once every 24 hours during the 4-d treatment period (day 1- 4). After the measurements were made, the plants were returned to the treatment chambers and re-injected with ethylene to satisfy the treatment concentrations. Plant height was recorded on 15 nongrafted and 15 grafted plants from each treatment group. The remaining 10 grafted and 10 nongrafted plants from each treatment chamber were used to monitor the maximum quantum efficiency of photosystem II (F_v/F_m) and leaf epinasty. An OS30p+ handheld chlorophyll fluorometer equipped with leaf clips was used to measure F_v/F_m on a single leaf from each replicate plant (Opti-Sciences, Inc. Hudson, NH, USA). The leaves were dark-adapted for 30 minutes prior to

measurement. Symptoms of leaf epinasty were quantified by measuring the percent change in leaf angle where the adaxial side of the third true leaf met the main stem.

Experiment 2: Seedling Growth

After the 4-d ethylene treatment, the plants were transported to the greenhouse environment and transplanted into nursery pots (6'' diameter x 5.6'' height) with Sun Gro Professional Growing Mix. The plants were arranged in a completely randomized design on the greenhouse bench. The plants were fertilized weekly (Jack's Professional LX water-soluble fertilizer 15-5-15 4Ca2Mg). Six grafted and six nongrafted plants from each of the ethylene treatment groups were destructively sampled on the day of transplanting (day 0) and on days 7, 14, and 21 after transplanting. On each sampling day, stem length, total leaf area and stem width were recorded. Stem length was measured from the soil surface to the apical meristem with a ruler stick. The leaf area was measured with an L-3100C Area Meter (LICOR, Lincoln, NE, USE). The stem diameter measurements were taken 1 cm above the cotyledons using a digital caliper (Husky Tools, Atlanta, GA). The shoot and root tissue of each sampled plant was dried at 60 °C in a laboratory oven for 48 hours to evaluate dry biomass. Plant compactness is reported for day 0 and was calculated by dividing dry shoot biomass by plant height (Meyer et al., 2017). Day 0 measurements were considered an evaluation of transplant quality and the subsequent sampling days (7, 14, and 21) were collected to monitor early growth.

Statistical Analysis

All data was analyzed using SAS software (SAS Studio 3.8, SAS Institute, Cary, NC). For the ethylene treatment experiment, F_v/F_m and leaf epinasty were analyzed using the MIXED procedure with DDFM=KR in the MODEL. A REPEATED statement was used to account for repeated F_v/F_m and leaf epinasty measures on individual plants over the four-day treatment

period. The fixed effects include ethylene treatments, graft status, and an ethylene treatment x graft status interaction term. For plant height, grafted and nongrafted plants were analyzed separately for the fixed effect of ethylene treatment with repeated measures because the plants had significantly different heights at the start of the experiment. Mean separation was carried out by a least significant difference test (LSD) test at $P<0.05$.

For the greenhouse experiment, growth measurements were analyzed separately for each sampling day and separately for grafted and nongrafted plants. The data was submitted to the GLIMMIX procedure. The mean separation was carried out with Bonferroni's test at $P<0.05$.

Results/Discussion

Symptoms of epinasty occurred in all plants within 24 h (day 1) when treated with 1 $\mu L \cdot L^{-1}$ and 10 $\mu L \cdot L^{-1}$ of ethylene (Fig. 2.1A) The degree of epinasty would characterize the plants as being highly sensitive to ethylene (Edelman and Jones, 2014). Epinasty was most severe in the oldest true leaves, which is consistent with literature (Abeles et al., 1992). The plants treated with 0.1 $\mu L \cdot L^{-1}$ were not statistically different than the control treatment for symptoms of epinasty, indicating that the concentration required to produce symptoms is between 0.1 $\mu L \cdot L^{-1}$ and 1 $\mu L \cdot L^{-1}$ (Fig. 2.1A) Epinasty was less severe in grafted plants than the non-grafted plants on day 1 ($P \leq 0.01$) and day 2 ($P \leq 0.05$) when treated with 10 $\mu L \cdot L^{-1}$ $\mu L \cdot L$-1of ethylene (Fig. 2.1A). The grafted plants also had a lower incidence of epinasty after the first day of exposure compared to nongrafted plants when treated with 1 $\mu L \cdot L^{-1}$ of exogenous ethylene (Fig. 2A).

A decrease in F_v/F_m can provide an early indication of plant stress from multiple abiotic stressors (Baker and Rosenqvist, 2004). Grafted and nongrafted plants treated with 1 $\mu L \cdot L^{-1}$ and 10 $\mu L \cdot L^{-1}$ of ethylene declined in F_v/F_m during the four-day treatment, while those treated with

0.1 μL·L-1[1] and the control remained constant (Fig. 2.1B). The total decline in F_v/F_m in nongrafted plants treated with 1 μL·L^{-1} and 10 μL·L^{-1} at the end of 4 days was 0.37 and 0.42, respectively. The total decline in grafted plants was 0.17 and 0.19 for the1 μL·L^{-1} and 10 μL·L^{-1} treated plants, respectively. Ethylene is not known to affect the photosynthetic apparatus in tomatoes directly, but a reduced light interception from leaf epinasty can inhibit photosynthesis (Woodrow and Grodzinski, 1989). One possible reason for the reduced photochemical efficiency in nongrafted plants is that they experienced greater degrees of leaf epinasty in the first two days than the grafted plants (Fig. 2.1 A). Alternatively, the grafted plants could have improved stress tolerance, which has been demonstrated in grafted plants subjected to abiotic stress such as heat stress (Rivero *et al.*, 2003) and water stress (Suchoff *et al.*, 2018).

Grafted plants did not significantly change in plant height across the four-day treatment (Fig. 2C). All nongrafted plants increased in plant height from day 0 to day 4 (Fig. 2.1C). The increase in plant height was 8.2%, 10.9%, 22.1%, and 24.6% following the treatments of 0 μL·L^{-1} 0.1 μL·L^{-1}, 1 μL·L^{-1}, and 10 μL·L^{-1} of ethylene. Exogenous ethylene typically results in the cessation of stem elongation (Abeles et al., 1992), but here we see contrary results among the nongrafted tomatoes. Plants in flooded conditions exhibit ethylene-mediated shoot elongation as adaption response and treatment of plants at saturating ethylene concentrations can mimic this response (Jackson, 2008). Stem elongation of tomato seedlings is a negative quality trait, as growers prefer compact transplants with thick stems (Lee et al., 2010).

One day after being removed from the treatment chambers, necrosis of the two oldest leaves and abscission of the cotyledons occurred in both grafted and nongrafted plants treated with 1 μL·L^{-1} and 10 μL·L^{-1} of ethylene. All symptoms of leaf epinasty improved in the upper canopy within 24 hours. The plants with ethylene damage were of poorer quality at the time of

transplanting, as observed by significantly lower leaf area, greater plant height, lower compactness, and lower shoot biomass (nongrafted only) compared to the control plants (Table 2.1). Stem diameter and root biomass were not impacted by ethylene treatment on day 0. Yet, the stem diameter of nongrafted plants treated with 1μL·L^{-1} μL·L-1and 10 μL·L^{-1} μL·L-1remained lower than control plants for the subsequent three sampling periods. Shoot biomass remained lower than the control for grafted and nongrafted plants that experienced ethylene damage for the first two weeks of growth. By day 21, shoot biomass was comparable across all treatments. Root biomass accumulation in nongrafted plants was also negatively impacted by the 1μL·L^{-1}and 10 μL·L^{-1} ethylene treatments on day 7 and day 14, but were similar to the control plants by day 21 (Table 1).

Although all ethylene-treated plants survived and early growth was not severely impacted, plants treated with 1 μL·L^{-1} and 10 μL·L^{-1} of ethylene had poorer overall visual quality. It is during postproduction of potted plants—such as during shipping and retailing—where exposure to high concentrations of ethylene (~1 μL·L^{-1}) could occur (Jones and Edelman, 2013). Propagators of high-quality grafted tomato seedlings should take precautions to prevent extended ethylene exposure or the promotion of endogenous ethylene in greenhouse operations and the postproduction supply chain. To our knowledge, this is the first experiment looking at the difference in ethylene sensitivity between grafted and nongrafted plants. These preliminary results suggest that grafted seedlings differ in response to exogenous ethylene compared to nongrafted plants, as indicated by better maintenance of F_v/F_m, and unaltered plant height. Further research needs to be conducted to confirm these results and understand the mechanisms of altered ethylene responses.

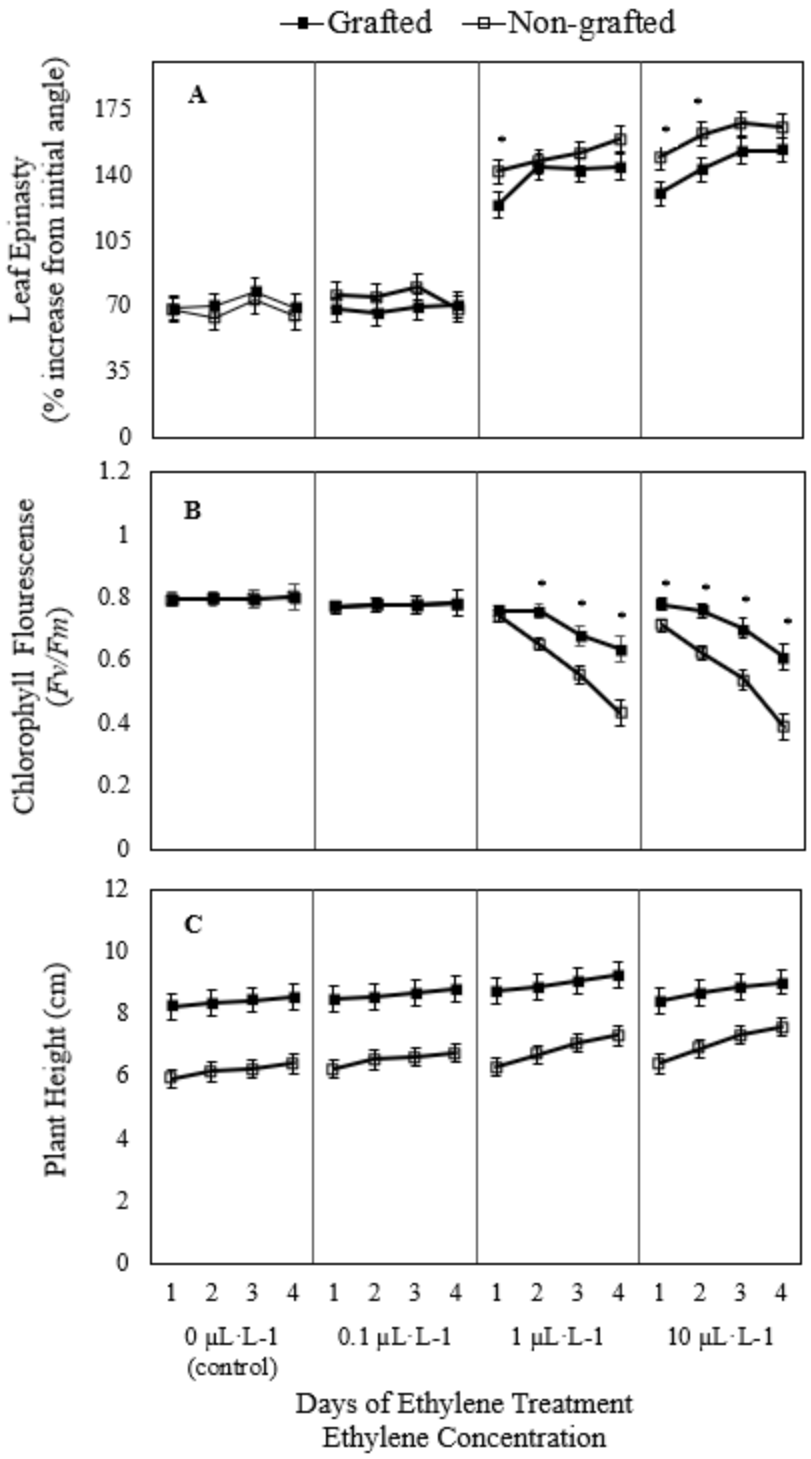

Figure 2.1. Leaf epinasty (A), Chlorophyll fluorescence Fv/Fm (B), and plant height (C) of grafted and non-grafted seedlings treated with 0 $\mu L \cdot L^{-1}$ (control), 0.1 $\mu L \cdot L^{-1}$, 1 $\mu L \cdot L^{-1}$, and 10 $\mu L \cdot L^{-1}$ ethylene over a 4-day treatment period. Points represent means of plant replicates for each parameter. Error bars represent the 95% confidence interval of the means at α=0.05. For leaf epinasty and Fv/Fm, * represents $P \leq 0.001$ of pairwise comparison between graft and nongrafted plants on a given day.

Table 2.1 Plant growth measurements of grafted and non-grafted 'Cherokee Purple' seedlings after 4-days of exogenously applied ethylene at the concentrations of 0 $\mu L \cdot L^{-1}$ (control), 0.1 $\mu L \cdot L^{-1}$, 1 $\mu L \cdot L^{-1}$, and 10 $\mu L \cdot L^{-1}$. Each value represents the mean of measurement obtained from 6 grafted and 6 nongrafted plants at each sampling period

	Grafted					Nongrafted				
	Control	0.1	1	10	p-value	Control	0.1	1	10	p-value
					Day 0[z]					
Leaf Area (cm^2)	42.53 a[y]	46.84 a	21.62 b	21.05 b	<.0001	50.82 a	59.11 b	16.50 c	17.50 c	<.0001
Stem (mm)	2.90	2.97	3.00	3.09	NS[x]	3.21	3.27	3.28	3.45	NS
Plant Height (cm)	9.70 b	9.32 b	10.12 b	11.13 a	<.0001	7.87 ab	7.70 b	8.35 ab	8.83 a	0.0273
Shoot Biomass (g)	0.18	0.17	0.15	0.13	NS	0.23 ab	0.29 a	0.20 b	0.19 b	0.0002
Root Biomass (g)	0.06	0.06	0.06	0.06	NS	0.09	0.11	0.09	0.08	NS
Compactness (g/cm)	1.86 a	1.79 a	1.44 ab	1.18 b	0.0009	2.96 a	3.70 b	2.36 b	2.15 b	<.0001
					Day 7					
Leaf Area (cm^2)	114.01	102.10	103.57	90.31	NS	133.62	151.48	116.67	107.08	NS
Stem (mm)	4.36	4.11	4.18	4.16	NS	4.92 a	4.82 a	4.61 ab	4.28 b	0.001
Plant Height (cm)	11.17	11.25	11.28	11.45	NS	9.70	9.98	10.67	10.52	NS
Shoot Biomass (g)	0.43 a	0.42 ab	0.37 ab	0.33 b	0.0143	0.55 b	0.63 a	0.46 c	0.43 c	<.0001
Root Biomass (g)	0.10	0.08	0.07	0.11	NS	0.17 ab	0.19 a	0.13 bc	0.11 c	0.0007
					Day 14					
Leaf Area (cm^2)	319.91 b	463.94 a	312.55 b	423.69 a	<.0001	377.66 ab	454.59 a	336.21 b	461.95 a	0.0010
Stem (mm)	6.35	6.47	6.58	6.61	NS	6.85 a	6.52 a	5.16 b	6.03 a	<.0001
Plant Height (cm)	15.67	16.77	16.40	16.84	NS	16.53	16.25	16.53	16.35	NS
Shoot Biomass (g)	1.97 a	1.92 a	1.62 b	1.55 b	0.0003	2.09 a	2.13 a	1.67 b	1.84 b	<.0001
Root Biomass (g)	0.33 b	0.44 a	0.27 b	0.33 b	0.0005	0.53 b	0.69 a	0.45 b	0.50 b	<.0001

						Day 21				
Leaf Area (cm^2)	667.73 b	792.25 ab	681.48 b	887.15 a	0.0054	771.25	877.97	839.99	859.65	NS
Stem (mm)	6.51 bc	7.6 ab	6.0 c	7.71 a	0.0003	7.66 a	7.17 ab	6.52 b	6.84 ab	0.0427
Plant Height (cm)	25.78 ab	23.18 b	28.6 a	23.32 b	0.0001	26.93	24.75	26.3	25.85	NS
Shoot Biomass (g)	5.08	5.75	4.84	5.73	NS	5.21	5.9	5.17	5.47	NS
Root Biomass (g)	0.62	0.59	0.52	0.59	NS	0.77	0.81	0.71	0.69	NS

[z]Day 0 is the day of transplanting and represents the quality of seedlings at time of transplanting.
[y]Means followed by the same letter are not significantly different at the α=0.05 significance level according to pairwise comparison with a Bonferonni *P*-value adjustment
[x]NS= Non-significant at the α=0.05 significance level.

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Chapter 3- Effect of heat stress on quality and early growth of grafted and nongrafted tomato seedlings during transportation

Abstract

The demand for grafted vegetable transplants has steadily increased in North America. Many vegetable growers purchase high-quality grafted transplants from specialty nurseries. These plants can be shipped long distances (2 to 5 days) to reach production regions. Unfavorable environmental conditions, such as high ambient temperatures during the shipment of containerized vegetable plants can impact transplant quality and early growth. The objective of this study was to investigate transplant quality impacts and early growth of grafted and nongrafted 'BHN-589' tomato (*Solanum lycopersicum*) plants when shipped under dark and non-ideal temperature conditions. 'Maxifort' was utilized as the rootstock, and grafted and non-grafted plants were placed in cardboard boxes in a growth chamber at the 3-4 true leaf stage and subjected to either 6, 12, 24, or 48 hours of exposure to 35°C within a 72-hr shipping simulation. Following the 72-hr treatment period, the seedlings were transplanted into pots and grown in the greenhouse for four weeks to monitor early growth. Nongrafted plants experienced significant succulent stem elongation (12% to 28%) during the simulated transportation, and the plants showed increased elongation with increasing durations under high temperatures. The maximal photochemical quantum yield of photosystem II (F_v/F_m) was also decreased in grafted and nongrafted plants from exposure to the high temperatures during simulated transportation. The declines in Fv/Fm increased with increased exposure time to the high temperature, but the total declines were more significant in nongrafted plants than grafted plants. Regardless of high temperature exposure, the shipped plants experienced reduced aboveground dry biomass

accumulation compared to the plants that remained in the greenhouse during the "shipping" period. Transplant compactness was also significantly reduced from transportation. These results suggest that initial transplant quality can be negatively impacted due to 72-hr shipping at above-optimal temperatures, which could reduce customer satisfaction. But, the early growth and development of the plants are not significantly affected.

Introduction

Grafting is a method of crop improvement that has historically been used in fruit-bearing vegetables to overcome soilborne diseases (Lee et al., 2010; Singh et al., 2017). This practice was commercialized in Japan and Korea in the 1960s to maintain yields in intensive, protective cropping systems where soilborne disease pressure was high (Kubota et al., 2008). Grafting of cucurbit and solanaceous crops is now practiced worldwide for many reasons, including to provide tolerance to abiotic stressors, improve plant vigor, enhance fruit quality, and increase yield and fruit marketability (Schwarz et al., 2010; Rivero, 2003; Soteriou and Kyriacou, 2015; Masterson et al., 2016). In the U.S., vegetable grafting has gained popularity over the last few decades, with tomato (*Solanum lycopersicum*) being the most commonly grafted vegetable (Ertle and Kubota, 2019). Hydroponic greenhouse operations make up the highest percentage of grafted tomato plants in the United States, where they are used to achieve higher yields via a more vigorous root system (Kubota et al., 2008). The phase-out of methyl bromide has also created a need for alternative soilborne disease management in tomato production. The use of grafted tomatoes represents a method that can meet management needs in areas where pathogen pressure is high (Lee et al., 2010; Louws et al., 2010). Other reasons for adopting this technology include the expansion of organic production, where using grafted plants can help overcome pests and diseases without agrochemicals (Barrett et al., 2012). Lewis et al. (2014) report that small

farmers are the fastest-growing segment of grafted plant users. The interest in low-yielding heirloom varieties and the increased use of high tunnels represent production practices where yields can improve with grafting in the absence of disease pressure (Louws et al., 2010; Moreno et al., 2019).

One barrier to the widespread adoption of vegetable grafting is the higher cost of grafted transplants than traditional transplants. Depending on the capacity and technology used by the grafting nursery, grafted plants can range from $0.59 to $1.25, whereas traditional transplants range from $0.13 to $0.51 per plant (Rivard et al., 2010). The economic benefits of using grafted plants can also depend on proper management and correct selection of rootstock and scion. Louws et al. (2010) note that using grafted plants for disease management is most successful when combined with other integrated pest management (IPM) tactics. Barret et al. (2012) expressed the importance of farmers choosing the appropriate rootstock and scion for a given production system and site to see yield and economic gains.

Commercial vegetable producers often purchase transplants rather than growing their own (Cantliffe, 2009). This trend is especially apparent with grafted plants due to the increased costs and labor required and the specialized nature of the propagation of grafted plants. The production of grafted seedlings in North America increased by 19% between 2015 and 2019 (Ertle and Kubota, 2019). However, there are still relatively few grafting nurseries in the U.S. Most grafted plants used in the U.S. are imported from Canada (Ertle and Kubota, 2019). Therefore, many grafting nurseries rely on shipping plants for long distances to reach growers, with durations up to four to five days (Pliakoni et al., *unpublished grower survey*).

High-quality seedlings have short, thick stems, large leaves, and well-developed root systems (Lee et al., 2010). However, transportation of containerized plants can lead to plant

quality concerns due to adverse environmental conditions. Extended darkness halts photosynthesis while respiration continues, and this can lead to a loss of chlorophyll and carbohydrate reserves (Ferrante et al., 2015; Starman et al., 2007). As temperature increases in dark storage, plant respiration and ethylene biosynthesis can also increase, resulting in leaf or flower drop and internode elongation of potted plants (Zhang and Zhou, 2013; Starman et al., 2007). Optimizing transportation and finding low-cost methods of transporting grafted transplants without quality deterioration will allow specialized grafting nurseries to reach more customers and expand the use of grafted vegetables among growers. The handling and shipping conditions of tomato transplants can directly impact transplant quality and post-transplant performance (Leskovar and Cantliffe, 1991; Risse and Moffitt, 1980). For instance, tomato transplants that were pulled and boxed had a lower shoot and root growth than plants that remained in trays during 72-hr storage at 20/28 °C (day/night). Mechanical stress during transportation can also stimulate an ethylene stress response and contribute to transplant shock in tomato plants (Agehara, 2020). Optimizing transportation and finding low-cost methods of transporting grafted transplants without quality deterioration will allow specialized grafting nurseries to reach more customers and expand the use of grafted vegetables among growers.

Low-temperature storage of containerized vegetable transplants is an effective strategy for slowing growth and maintaining seedling quality during dark storage or transportation (Kubota and Kroggel, 2004; Leskovar and Cantliffe, 1991; Sato et al., 1999). Recommended low-temperature storage varies for tomato transplants based on storage duration and physiological age. Mature tomato seedlings with visible flowers can be stored in the dark at 6 to 13 °C without quality loss for up to four days (Kubota and Kroggel, 2006). Kubota and Kroggel (2006) found that low temperatures helped prevent succulent stem elongation, flower drop, and

loss of early yields due to irregular truss development. Prevention of flower drop is likely mediated by preventing ethylene biosynthesis and accumulation during transportation with cooler temperatures. Shipping under ambient temperatures (18 °C) with the application of 1-MCP can similarly prevent flower drop and abnormal truss development (Kubota and Kroggel, 2011).

Seedlings for open-field and high tunnel cultivation are typically shipped before the plants have first flowers—around one or two weeks after the plants have healed from grafting. Risse, Moffitt, and Bryan (1979) recommend storing containerized tomato seedlings at 10 to 12.7 °C for a maximum of ten days to limit growth during storage, maximize survivability, and maintain post-transplanting growth. Kwack et al. (2016) report that the optimal temperature and duration to ship grafted tomato seedlings (272-hrs after grafting) is 6 °C for up to six days. Higher temperatures (18 °C) increased stem elongation, decreased chlorophyll content, and decreased percent plant survival after transplanting compared to those shipped at 6 °C (Kwack et al., 2016).

Despite the ability of refrigeration to mitigate quality deterioration during long-distance transportation, a survey conducted by the authors in 2018 indicates that refrigeration is not industry standard. Environment control during transport can be cost-prohibitive—especially for small orders, often boxed and shipped through commercial carriers such as FedEx (Ricardo Hernandez, personal communication). Shipping during warmer months can lead to higher than ambient conditions in boxes or trailers. Additionally, temperatures inside vehicles can be warmer than outdoor temperatures due to the heat transfer from the road pavement and the trailer. Temperatures at the canopy level can also remain higher than the trailer environment, depending on how densely packed the plants are (Risse, Moffitt and Bryan, 1979).

It is well-documented that tomatoes undergo heat stress at temperatures above 35 °C (Rivero et al., 2001; Wahid et al., 2007). Symptoms of heat stress are dependent on intensity, duration, and the plant's growth stage (Wahid et al., 2007). The reproductive or flowering stage is more sensitive to heat stress in tomatoes than the vegetative seedling phase. (Foolad, 2005; Wahid et al., 2007; Zhou et al., 2017). At the seedling stage, heat stress can reduce photosynthesis by impacting RUBISCO activity and the function of photosystem II (Camejo et al., 2005; Zhou et al., 2017). A decrease in photosynthetic activity can also disrupt the carbon balance in tomato seedlings (Zhou et al., 2017).

Thermotolerance can differ across genotypes of a given species, and this has been demonstrated in tomato (Camejo et al., 2005; Sato et al., 2004; Zhou et al., 2017). Zhou et al. (2017) indicated that heat sensitivity could differ among tomato cultivars, with the heat-tolerant cultivar 'LA1994' remaining photosynthetically unaltered during a 4-day treatment at 35 °C under a regular photoperiod. Grafting tomato scions to vigorous rootstocks may also provide thermotolerance by maintaining better growth and biomass under prolonged heat stress conditions than nongrafted plants (Abdelmageed and Gruda, 2009; Rivero et al., 2003). It is less clear if the effects of short-term exposure to high temperatures on young grafted tomato seedlings under dark conditions—such circumstances that plants may experience during transportation without environmental control. It is also unclear if grafting can provide tolerance to these adverse conditions. The objective of this study is to examine the effects of various exposure times to a high temperature (35 °C) on grafted and nongrafted tomato seedlings during a 72-h simulated transportation experiment. Assessing the immediate seedling quality impacts and the post-transplanting growth and development will allow for a better understanding of the impact of non-ideal shipping temperatures on grafted tomato transplants.

Materials and Methods

Two separate experiments were performed to accomplish the above-mentioned research objectives. First, grafted and nongrafted plants were treated with increasing durations of a high temperature during a simulated transportation experiment, and observations were made about the quality and physiological status of the plants. Next, the grafted and nongrafted plants were transplanted and grown in a quonset-style greenhouse to observe growth and performance following the treatment period. The whole experiment was performed twice (2020 and 2021).

Plant materials

Grafted and nongrafted tomato transplants (*Solanum lycopersicum* L.*)* were propagated and grafted in a Quonset-style greenhouse at the Kansas State University Olathe Horticulture Research and Extension Center, located in Johnson County, KS (38.884347, -94.993426). The hybrid tomato 'BHN 589' (BHN Seed; Immokalee, FL) was used for the grafted and nongrafted groups. 'Maxifort' was used as the rootstock (DeRuiter Seeds; St. Louis, MO). Seeds were sown in Fafard Germinating Mix in 30 cm by 30 cm flat trays (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). Ten days after sowing, plants were transplanted into 50-cell propagation trays filled with Metro-Mix 852 Professional Growing Mix (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). Greenhouse temperatures were maintained at 27 ± 5 °C (day) and 17 ± 2 °C (night). Approximately 17 days later, the plants were grafted using the splice/tube grafting method outlined by Rivard and Louws (2011). The plant were placed in a healing chamber located in a second greenhouse environment where temperatures were maintained at 25± 5°C (day) 20± 2 °C (night). The chambers consisted of frames covered with a polyethylene film and 55% shade cloth and were equipped with a cool-mist humidifier. The plants remained in the healing chambers for ten days. After healing, the plants were grown in the

original greenhouse environment for one week before beginning the simulated transportation experiment. The nongrafted 'BHN 589' plants were sown two weeks after the grafted group, so all plants were at the same maturity at the start of the experiment. Watering was withheld for 24 hours before the start of the experiment. All plants had 3 to 4 true leaves at the beginning of the experiment.

Simulated high-temperature transportation

High-temperature treatments. The 72-hour simulated transportation experiment took place from February 28th to March 3rd in the first full replication of the experiment (2020) and from March 22nd to March 25th in the second replication (2021). In 2020, groups of nine grafted and nongrafted plants were placed into separate cardboard boxes (8 L x 8 W x 10 H). Each box was an experimental unit assigned to one of the treatments: 0, 6, 12, 24, or 48 h of exposure to 35 °C. In 2021, eight plants were used per experimental unit. The exposure duration to 35 °C will be referred to as exposure time treatments. Four replications of grafted and nongrafted plants were used for each exposure time treatment in each year. A non-shipped control group was also included in the experiment—thus, four groups of nine grafted and nongrafted plants were left in the original greenhouse environment for the 72-hour treatment period. The experiment took place in four environmental chambers with all light excluded (ThermoFisher Scientific Inc., Asheville, NC, USA). Two chambers were set to 25 °C to act as an ambient shipping temperature, and two chambers were set to 35 °C for the high-temperature treatment. The experiment was arranged in a randomized complete block design, with environmental chambers as the blocks. All containers were placed in the 25 °C chambers at the start of the experiment and remained there for 24 hours. Containers were randomized on the shelves of the chambers. The

containers were then moved to a 35° C treatment chamber to correspond with the proper exposure time treatment—6 h, 12 h, 24 h, and 48 h. All exposure time treatments ended the 72-hour shipping simulation at the same time. Each chamber was monitored for temperature and relative humidity with EL-21CRF-2-LCD temperature data loggers (Lascar Electronics, Erie, PA, USA). Temperature probes were also placed inside 1-3 boxes per treatment to account for temperature and humidity inside the boxes.

Stem length. Before the 72-hr experiment, six plants from each container were measured for stem length. Length measurements were recorded by measuring from the soil surface to the apical meristem with a ruler stick. This measurement was repeated on the same six plants from each box immediately after the 72-hr simulated transportation.

Chlorophyll fluorescence (F_v/F_m). The maximum quantum efficiency of photosystem II (F_v/F_m) was measured on three randomly selected plants from each box before being placed into the environmental chambers for the treatment period. The same three plants from each container were measured after the 72-hour treatment period. Chlorophyll fluorescence was measured on the second true leaf from each plant using an OS30p+ handheld chlorophyll fluorometer equipped with leaf clips (Opti-Sciences, Inc. Hudson, NH, USA). The plants were dark-adapted for 30 min at room temperature before measurement.

Post-transplanting growth

Greenhouse experiment. The grafted and nongrafted plants were returned to the greenhouse after the 72-h transportation simulation. Plants remained on the greenhouse bench for 24 hours and were then transplanted into nursery pots (6.5'' diameter x 6.5" height) with Sun Gro Professional Growing Mix. The plants were placed on greenhouse benches in a complete randomized design in both 2020 and 2021. Table 3.1 provides the environmental conditions

during the post-transplanting growth for both years. All plants were fertilized weekly (Jack's Professional LX Water-soluble fertilizer 15-5-15 4Ca 2Mg). Two weeks after transplanting, bamboo stakes were placed in the pots to support the plants.

Table 3.1 Greenhouse environmental conditions during the four-week growth period following simulated transportation for repeated experiments in 2020 and 2021.

Dates	Temperature °C
2020	
March 4- March 10	24 ± 5[z]
March 11- March 17	24 ± 2
March 18- March 24	24 ± 2
March 25-March 31	26 ± 3
2021	
March 30- April 5	24 ± 8
April 6- April 12	24 ± 7
April 13- April 19	23 ± 7
April 20- April 26	25 ± 8

[z]Values represent weekly means ± the standard deviation from measurements taken every 15 minutes

Data collection. On the day of transplanting and seven days post-transplanting (day 7), four randomly selected grafted and nongrafted plants were destructively sampled from each exposure time treatment. Plant height, stem diameter, plant compactness, shoot/root ratio, leaf area, shoot dry weight, and root dry weight was measured as an assessment of transplant quality and early growth. The plant height was measured from the soil surface to the apical meristem. Stem diameter measurements were taken 1 cm above the cotyledons using a digital caliper (Husky Tools, Atlanta, GA). Each plant was cut at the soil surface and defoliated. Total leaf area was measured with an LI-3100C Area Meter (LICOR, Lincoln, NE, USA). The plant shoots and roots were dried in a laboratory oven at 65 °C for 48 hours and then weighed on a digital scale for the collection of dry biomass. Plant compactness was calculated by dividing the dry shoot

biomass by the plant height as described in (Meyer et al., 2017). The plants were destructively sampled for three additional weeks (day 14, 21, 28) for the collection of dry biomass. The number of days (after transplanting) to anthesis was counted in 2020, only. The number of flower buds, partially opened flowers (presence of any yellow), and fully open flowers (anthesis and post-anthesis) were counted on day 21 and day 28 in both years.

Data Analysis

All data were analyzed using SAS software (SAS Studio 3.8, SAS Institute, Cary, NC). The simulated transportation experiment was arranged in a randomized complete block design, with environmental chambers as the block. Each repetition (year) was also considered a random blocking factor. The experimental unit was a box of 8-9 plants, and the sampling unit was a subset of plants within each box.

The *Fv/Fm* results were analyzed in two different ways. To determine if the grafted and nongrafted plants had significantly different *Fv/Fm* values after the exposure time treatments compared to the initial values, the data were analyzed as a 2-way treatment structure with measurement day (before vs. after the treatment period), exposure time treatments, and an interaction term as the fixed effects. The data was subjected to a MIXED procedure with DDFM=Kenward Roger in the MODEL statement and a REPEATED statement to account for repeated measurements on the same plants at two different time periods. The blocking factors and treatment interactions with the blocking factors were included in the RANDOM statement. Grafted and nongrafted plants had significant differences in Fv/Fm at the start of the experiment for these parameters. Therefore, they were analyzed separately but similarly. All mean separation was carried out using a Bonferroni p-value adjustment to account for multiple comparisons.

The percent change in *Fv/Fm* and plant height were calculated as (after treatment period – before treatment period ÷ before treatment period) *100. The percent change was used as the response variable and analyzed with the GLIMMIX procedure with graft status, exposure time treatments, and an interaction term as the fixed effects. The blocking factors and interactions with the blocking factors were included in the RANDOM statement. All mean separations were carried out using a Bonferroni p-value adjustment to account for multiple comparisons.

Transplant quality and early transplanting growth parameters (plant compactness, aboveground biomass, and root biomass, height, stem diameter, shoot/root ratio, and leaf area) were analyzed separately for grafted and nongrafted plants due to significant differences in some of the morphology parameters at the start of the experiment. For assessment on the day of transplanting and day 7, each parameter was subjected to the GLMMIX procedure with exposure time treatment and replication year as a fixed effect. The flower count data was natural log-transformed to improve the normality of the data and the overall model fit and then analyzed in the same manner as previously described.

To assess the treatment effects on plant growth, the total biomass (shoot dry biomass + root dry biomass) from each measurement day were natural log-transformed and subjected to a linear regression analysis using the MIXED procedure with DDFM=KR in the model statement. Graft status, exposure time treatments, measurement day, and all interaction terms were included in the model as fixed effects. The two years were analyzed separately because there were significant three-way and four-way interactions effects with year. The ESTIMATE statements were used to perform t-tests on treatment differences in the rate of growth (slopes) among the fixed effects of heat treatments, graft status, and an interaction term. The predicted biomass

values from the regression analysis were used in a REG procedure to assess the overall statistical model fit.

Results

Internal shipping container environment

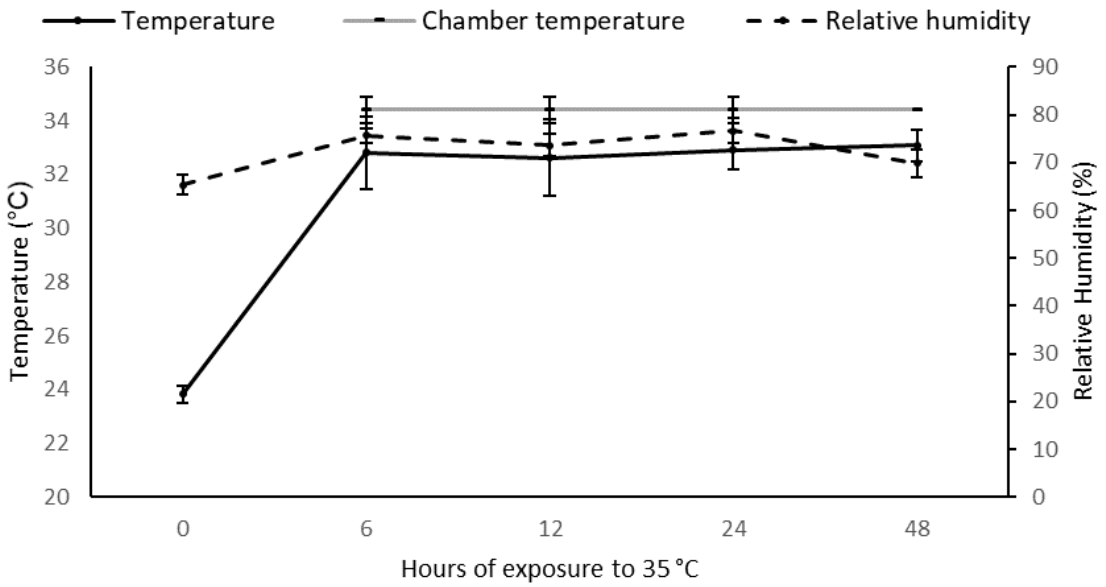

Figure 3.1. Environmental conditions inside shipping containers during 72-hr. treatment. Values represent mean temperature and relative humidity ± SD from 1-2 boxes from each treatment across both years of the experiment and in both environmental chambers**.**

Chlorophyll fluorescence

The F_v/F_m ratio of grafted and nongrafted plants was not significantly reduced from the initial conditions in the control plants that were held at 25 °C for the 72-h treatment period (Table 3.2). The exposure threshold that led to a significant reduction in F_v/F_m from the initial conditions was 6 h for both grafted and nongrafted plants (Table 3.2). There was a significant grafting effect ($P \leq 0.01$) and exposure time treatment effect ($P \leq 0.01$) on the percent reduction in F_v/F_m (Table 3.3). The interaction term was not significant for this parameter, so only the main effects are considered. The reduction in F_v/F_m increased with longer exposure to the high temperature, but only the 24 h and 48 h durations led to significant decreases compared to the control plants that remained at 25 °C for the treatment period *(*$P \leq 0.01$, $P \leq 0.001$, respectively) (Table 3.3). Overall, there was a greater percent reduction in F_v/F_m in nongrafted plants at all exposure time treatments than the grafted plants ($P \leq 0.01$, Table 3.3).

Table 3.2 The change in *Fv/Fm* in grafted and nongrafted tomato plants exposed to increasing durations to 35 °C during a 72-hr. treatment period**.**

Treatments[z]		Before Transportation *Fv/Fm* ratio	After Transportation *Fv/Fm* ratio	% change	P-value[x]
Grafted	0 h	0.7902[y]	0.7925	0.53	ns
	6 h	0.7844	0.7701	-1.57	0.0110
	12 h	0.7814	0.7631	-2.35	0.0009
	24 h	0.7754	0.7554	-2.63	<.0001
	48 h	0.7770	0.7359	-5.32	<.0001
Nongrafted	0 h	0.8011	0.7978	-1.73	ns
	6 h	0.7958	0.7736	-3.88	<.0001
	12 h	0.7997	0.7702	-4.57	<.0001
	24 h	0.8012	0.7685	-5.57	<.0001
	48 h	0.7964	0.7632	-6.46	<.0001

[z]Treatments include graft status and duration of exposure time to high temperature (0 h, 6 h, 12 h, 24 h, 48 h) during simulated transportation.
[y]Values represent LSMEANS (n=24)

[x]P-value is the significance level of the pairwise comparison between the initial conditions and post-treatment conditions of *Fv/Fm* values for each exposure time treatment with a Bonferonni adjustment where α=0.05.

Table 3.3 Percent change in *Fv/Fm* following 72-hr treatment period from the main effects of exposure time treatments and graft status

Main effects	% change *Fv/Fm*
Exposure time treatments	
0 h	-0.60 a[z]
6 h	-2.72 ab
12 h	-3.46 ab
24 h	-4.10 b
48 h	-5.89 b
P-Value[y]	0.0026
Graft status	
Non-grafted	-4.44 b
Grafted	-2.27 a
P-Value	0.0036

[z]Percent reduction of main effect LSMEANS followed by the same letters are not significantly different (P>0.05) according to pairwise comparisons with a Bonferroni adjustment.
[y]Significance level of overall ANOVA F-test

Stem elongation

There were significant main effects of graft status ($P \leq 0.01$), exposure time treatments ($P \leq 0.01$) and interaction effect ($P \leq 0.0001$) on the percent stem elongation. The percent stem elongation of the control plants that were not shipped represents the normal growth of seedlings that remained in the greenhouse during the 72-h treatment period (Fig 3.2). The nongrafted plants elongated significantly more than grafted plants under all exposure time treatments. Nongrafted plants generally elongated to a greater extent when exposed to longer durations of the high temperature, with total increases ranging from 12% to 28% (Fig 3.2). Grafted plants under all of the exposure time treatments were not significantly different from the greenhouse control group.

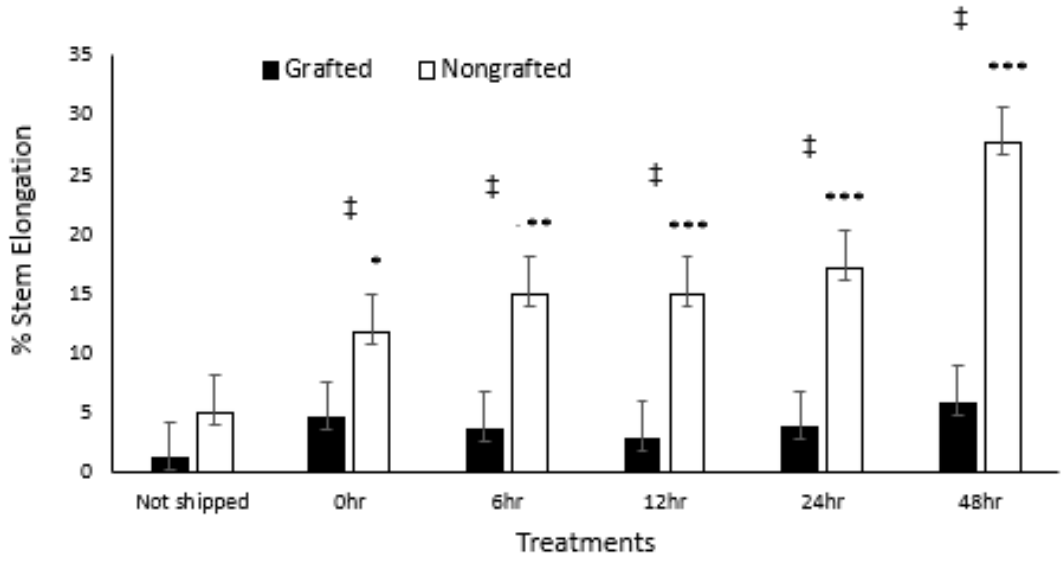

Figure 3.2 The effect of treatments and graft status on stem elongation during the 72-hr. treatment period. Treatments are the duration of time exposed to the high temperature during a 72-h treatment period. Each bar represents the least squares mean ± the 95% CI for 6 sampled plants from four experimental unit replication across the two experiments (n=24). Non-shipped controls represent the normal growth of seedlings in the greenhouse. * denotes *P*-value ≤.05, *** , P-value <0.01, ** P-value <0.001 for significant differences of treatment groups to the non-shipped control treatment of same graft status. ‡ denotes *P*-value ≤.05 for significant difference between grafted and nongrafted plants at each exposure time treatment. All post-hoc tests were performed with a Bonferroni p-value adjustment**.**

Transplant quality and early growth

Table 3.4 provides plant growth parameters from 1 day after the 72-hr. transportation simulation (at the time of transplanting) and after 7 days of growth in the greenhouse. Regardless of exposure time treatment, plant compactness was significantly reduced by an average of 32% in grafted plants and 33% in nongrafted compared to the non-shipped control plants ($P \leq 0.01$ for grafted, $P < .0001$ for nongrafted, Table. 3.4). The dry shoot weight of grafted plants from all exposure time treatments was also reduced by 32% compared to the non-shipped plants, however, the reduction was only significant in the 0 h., 6 h, and 48 h treatments ($P \leq 0.05$). The dry shoot biomass of nongrafted plants from all exposure time treatments were reduced by an

average of 28% compared to the non-shipped control plants at the time of transplanting ($P<.0001$). The duration of exposure to the high temperature did not influence the degree of reductions in plant compactness and dry shoot biomass. In nongrafted plants only, the shoot:root ratio was significantly reduced (27%) in the treatment exposed to 6 h of the high temperature ($P\leq0.05$). The exposure time treatments did not impact the parameters of stem diameter, dry root biomass, leaf area (2021 only), and shoot:root (grafted only) of plants on the day of transplanting. There were few significant differences among the exposure time treatment groups for any growth parameter on day 7. The nongrafted, non-shipped control plants had a significantly lower leaf area than the other treatments and shorter plant heights compared to the 0 h and 48 h treatments.

Figure 3.3 provides the rate of growth of the greenhouse-grown plants following the simulated exposure treatments. There were no significant graft x exposure time treatment effects on the growth rate, so grafted and nongrafted plants are considered together in the growth rate analysis. In 2020, the growth rates of plants exposed to 6 h and 12 h were statistically faster compared to the non-shipped plants. In 2021, all exposure time treatments had statistically similar growth rates. The overall fits of the log-linear regression plant growth models were sufficient to describe the growth rate of the plants. The models accounted for 97% of the data variation in 2020 and 94% in 2021 (Fig. 3.4).

Graft status and exposure time treatments did not impact the days to anthesis or the number of flowers counted on day 21 or 28 (Fig. 3.5) in 2020. Only floral buds were counted on day 21. On day 28, floral buds, partially opened flowers, and fully opened flowers were present. There was no statistical difference between treatments for the flower count data in 2021 (data not shown).

Table 3.4 Growth parameters at time of transplanting and 7 days after transplanting for grafted and nongrafted tomato plants following 72-hr. simulated exposure time treatments in 2020 and 2021.

Treatment[z]		Compactness (mg/cm)	Stem diameter (mm)	Leaf area (cm^3)	Plant height (cm)	Dry Shoot Biomass (mg)	Dry root biomass (mg)	Shoot:root ratio
Day of transplanting								
Grafted	Not shipped	27.5 a	3.2885	45.1	8.4	228.4 a	53.1	4.5
	0hr (25 °C)	17.5 b	2.9755	55.3	8.6	144.5 b	46.3	3.2
	6h	18.6 b	3.1205	48.1	8.2	147.5 b	43.8	3.5
	12h	20.4 ab	3.231	61.8	8.2	167.6 ab	47.5	4.0
	24h	18.9 b	3.3459	64.1	9.1	171.1 ab	47.5	3.5
	48h	18.1 b	3.2655	58.8	8.6	150.9 b	50.0	3.2
	P-Value[x]	0.0047	ns	ns	ns	0.039	ns	ns
Nongrafted	Not shipped	42.1 a	2.5848	42.6	4.8	209.1 a	54.2	4.2 a
	0h (25 °C)	31.7 b	2.7322	54.6	5.1	161.9 b	59.3	3.1 ab
	6h	25.9 b	2.585	39.3	5.0	131.0 b	55.3	3.0 b
	12h	29.2 b	2.5381	44.1	5.5	159.4 b	50.7	3.5 ab
	24h	26.2 b	2.6407	34.6	5.2	132.7 b	42.5	3.2 ab
	48h	28.3 b	2.8528	35.7	6.0	166.8 b	65.7	3.1 ab
	P -Value	<.0001	ns	ns	ns	<.0001	ns	0.034
Day 7								
Grafted	Not shipped	54.9	4.5 ab	102.7	8.6	479.9	82.5	5.6
	0h (25 °C)	55.4	5.0 a	137.3	9.9	535.7	98.2	5.6
	6h	53.0	4.8 ab	123.6	9.4	496.4	99.9	5.3
	12h	51.3	4.4 ab	384.0	9.1	467.8	96.6	4.7
	24h	47.4	4.6 ab	123.4	10.2	472.1	85.7	5.4
	48h	47.2	4.2 b	133.7	9.9	449.1	95.7	5.2
	P -Value	ns	ns	ns	ns	ns	ns	ns
Nongrafted	Not shipped	58.1	4.4	62.3 b	6.1 b	378.3	108.8	3.8
	0h (25 °C)	59.7	4.7	95.6 a	7.5 a	434.5	125.6	3.6
	6h	58.9	4.4	102.6 a	7.1 ab	417.1	112.9	3.7
	12h	56.7	4.4	101.3 a	7.1 ab	414.7	120.6	3.6
	24h	55.9	4.3	97.2 a	6.7 ab	381.0	106.8	3.7
	48h	54.1	4.3	109.8 a	7.4 a	418.8	116.4	4.1
	P -Value	ns	ns	0.017	0.023	ns	ns	ns

LSmeans in each column for a given graft status that are followed by the same letter are not significantly different (P>0.05) according to pairwise comparison with a Bonferonni p-value adjustment
[z]Treatments include graft status and duration of exposure to high-temperatures (0 h, 6 h, 12 h, 24 h, 48 h) and a control group that remained in the greenhouse for the 72-h treatment period.
[y]Values represent LSMEANS of 4 experimental units (individual plants).
[z]P-value is the probability value for the overall ANOVA F-test where α=0.05.

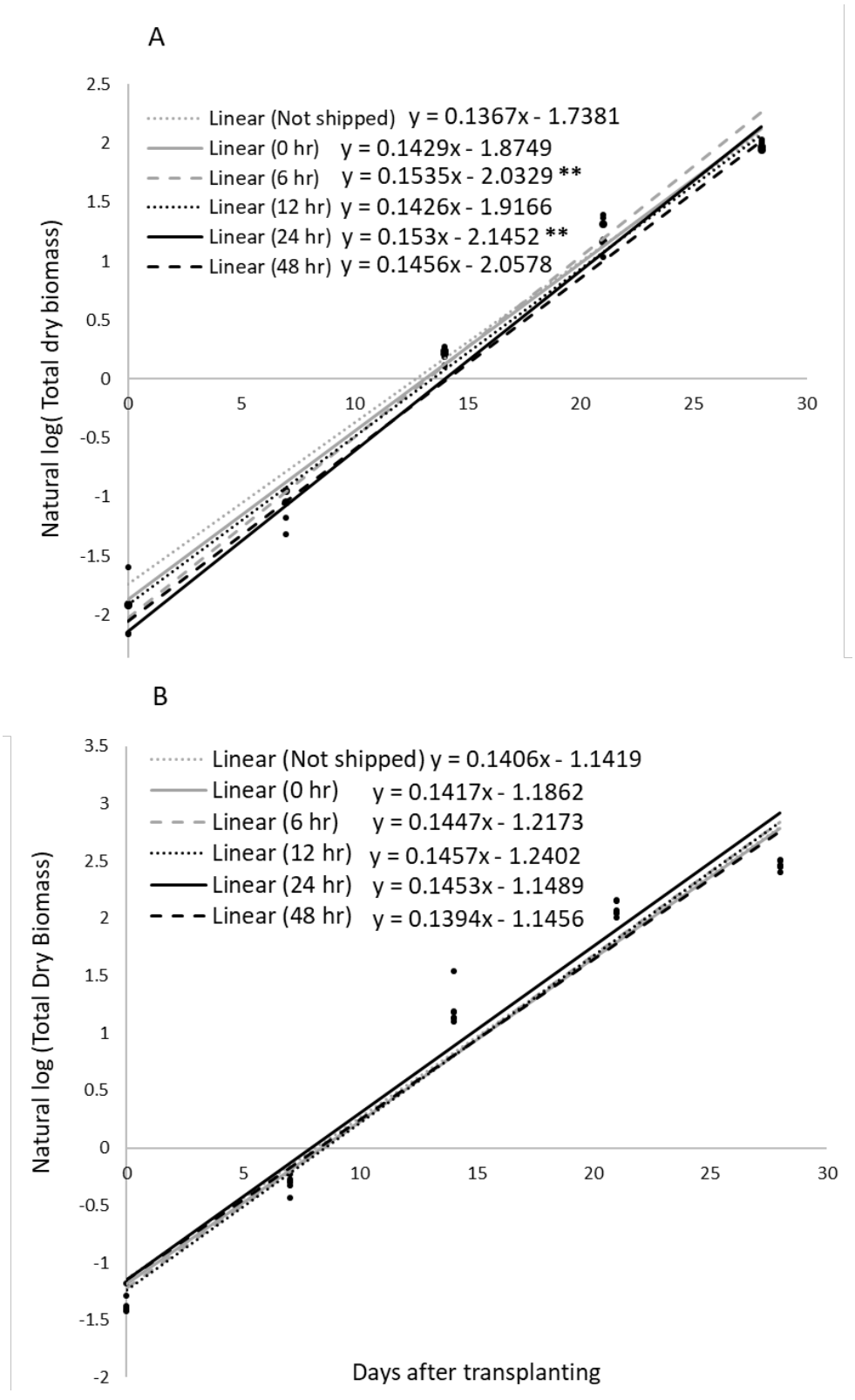

Figure 3.3 The log-linear growth rate of plants from each exposure time treatment in (A) 2020 and (B) 2021. The effect of graft status and exposure time treatment were additive, so only exposure time treatments are considered. Each sampling day represents the natural log of the total dry weight LSmeans of 8 plants from each treatment group. ** represent a statistical difference between the slope of the exposure time treatment and the not-shipped control plants according to a t-test where $P \leq 0.01$.

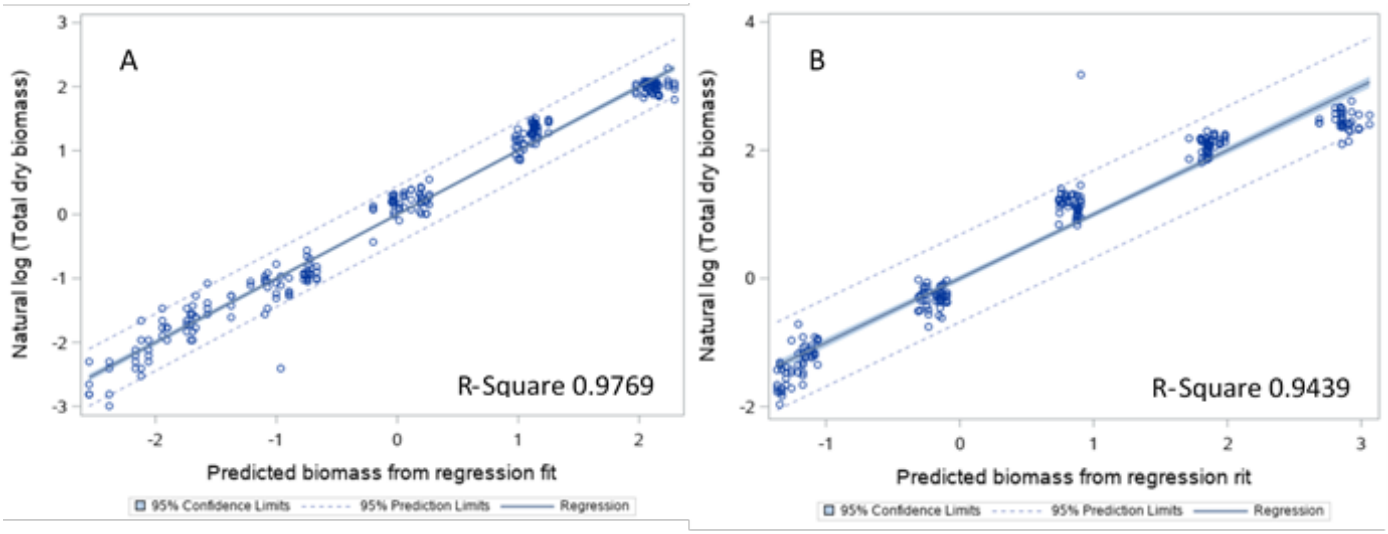

Figure 3.4 Overall fit of the log-linear growth models from (A) 2020 and (B) 2021. The predicted ln(biomass) is plotted with the true ln(biomass) values along with a 95% confidence interval bands (dotted lines).

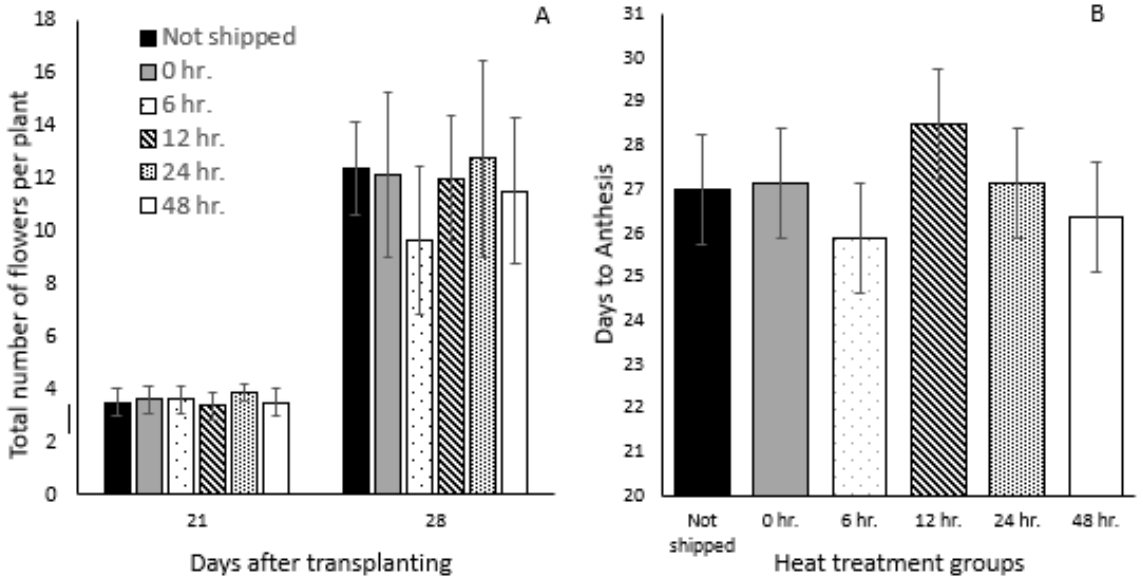

Figure 3.5 (A) Total flower count per plant from each exposure time treatment group. Total flower count is the sum of all floral buds, partially opened flowers, and fully opened flowers. Each bar represents the mean of 8 replicate plants ± the standard deviation. There was no statistical difference regarding total flower count between exposure treatments according to a

mixed model fitted with natural log transformed count values. (B) The number of days to anthesis (days after transplanting) from each exposure time treatment group. Each bar is the mean of 8 plants ± the standard error of the mean from fitting a mixed model. There was no statistical difference regarding the number of days to anthesis between the exposure treatments. The effect of graft status was not significant for total flower count or the days to anthesis, so the data was pooled and only the effect of exposure treatments was considered. Data is from the experiment conducted in 2020 only**.**

Discussion

The air temperature within a box of plants remained 1.6± 0.20 °C lower than environmental chambers, which were 34.4 ±0.5 °C. Therefore, the plants experienced moderate heat stress, which is not expected to cause the full extent of biochemical damage (Sato et al., 2004), but could still inhibit normal plant growth and photosynthetic capacity (Sharkey, 2005). It should be noted that water stress was not controlled in this study, and it can be difficult to separate water stress from heat stress under such conditions. Water was withheld from the plants one day before the experiment because plants are often hardened through reduced watering prior to transportation.

The F_v/F_m ratio is a good tool to detect early plant stress and can be used as a screening tool for heat tolerance at the seedling stage (Zhou et al., 2015). In this trial, the moderate heat stress exposure treatments had similar effects on grafted and nongrafted plants. Yet, the overall decrease in F_v/F_m was more significant in nongrafted plants than grafted plants. Better maintenance of F_v/F_m under heat stress could indicate that the grafted plants had improved thermotolerance (Zhou et al., 2017b; Zhou et al., 2015). The initial values of F_v/F_m were lower for grafted plants at the start of the experiment, which has been shown to indicate poor graft compatibility (Goto et al., 2013). However, the graft combination used in this study has proven compatibility (Loewen et al., in press), and the plants did not show signs of incompatibility after transplanting in this experiment, so it is likely that the reduced initial F_v/F_m values of the grafted

plants are from the stress of grafting and healing. Moderated stress is known to improve secondary stress response. For example, polyethylene glycol-induced drought stress can improve the chilling tolerance of tomato seedlings by increasing the antioxidant enzyme activity, abscisic acid, anthocyanin accumulation, potassium (K^+) and proline content (Ghanbari and Sayyari, 2018). Therefore, the better maintenance of F_v/F_m in grafted plants could result from the moderate stress experienced during grafting and graft union formation.

F_v/F_m declined with increasing time exposed to the high temperature in this study. This signifies the importance of the duration of heat stress on the physiological damage to tomato seedlings. Zhou et al. (2017) found a similar relationship between the length of exposure to high temperatures (36/28 °C) and a decline in F_v/F_m of nongrafted 'Aromata' tomato seedlings grown under illumination. Under laboratory conditions using tomato leaf discs, the decline in F_v/F_m was quadratic with increasing temperatures (20 °C to 40 °C) for a 60-minute treatment period (Willits and Peet, 2001). In darkness, the damage to photosystem II is primarily due to the inactivation of the oxygen-evolving complex of photosystem II, as demonstrated in tobacco plants treated at high-temperatures for 4 h (Yang et al., 2007). Yang et al. (2007) showed that when the heat was applied in the dark versus the light, there was less PSII inhibition and greater recovery of PSII. In this study, 24 h after the treatment period and after returning the plants to the greenhouse, there were no significant differences in F_v/F_m among any of the exposure time treatment groups or between grafted and nongrafted plants. Also, moderate heat stress may inhibit the repair mechanism of PSII from reactive oxygen species (ROS), but not the PSII reaction center itself (Nath et al., 2013; Yang et al., 2007). Therefore, it can be concluded that the physiological damage to tomato seedlings from moderate heat stress in the dark is temporary.

Succulent stem elongation is considered a negative trait for vegetable transplants and is a common plant response from dark storage (Heins et al., 1992; Kubota and Kroggel, 2006). Higher storage temperatures also increase elongation in bedding plants and tomato transplants (Heins et al., 1992; Kubota and Kroggel, 2006; Kwack et al., 2016). This is consistent with the results of the nongrafted tomato seedlings in this study, where stem elongation increased with increasing durations under the high-temperature treatment. However, grafted transplants did not significantly elongate from initial plant heights under any of the exposure treatments. It is possible that the rootstock provided stress tolerance to the plants, thus preventing significant succulent elongation. Sato et al. (2004) demonstrated that water-stressed cabbage plugs did not elongate as much as non-stressed plants during dark storage. Plants pre-conditioned to stress can better tolerate oxidative stress through improved enzyme and non-enzyme antioxidant production, phytohormone responses, and osmolyte production (Ghanbari and Sayyari, 2018; Zhang et al., 2018). Also, certain tomato rootstocks have also been shown to have improved antioxidant responses under abiotic stress (Zhang et al., 2019; Zhang et al., 2021). Rivero et al. (2003) found that grafted tomatoes could better maintain biomass under heat stress (35 °C) than nongrafted plants, but this was not due to enhanced antioxidant response. Rather, the rootstocks provided better tolerance to the stress. The improved tolerance in grafted plants in this investigation could be a direct effect from the rootstock or a result of stress pre-conditioning from the grafting process itself.

Our results showed that shoot biomass was significantly reduced in both grafted plants and nongrafted plants compared to the non-shipped control plants from highest exposure time treatments. This result is consistent with results from dark-stored eggplants plugs (Kubota et al., 2002). There were no clear trends on the effect of shoot dry matter reduction and exposure to

moderate heat stress, so darkness and ambient temperatures were likely the contributing factor in dry shoot biomass loss. The reduction in dry shoot biomass also contributed to the reduced compactness values of grafted plants. Compactness is a function of plant height and dry shoot biomass and can be a good indicator of high-quality grafted transplants (Ngoc Thang et al., 2013; Meyer et al., 2017). For nongrafted plants, succulent elongation and lower dry shoot biomass resulted in a lower compactness value. Similarly, Sato et al. (2004) and Sato et al. (1999) observed that dark-stored cabbage plugs had reduced starch content and elongated stems, indicating that starch was used for succulent elongation during dark storage. A reduction in dry matter is often attributed to a carbohydrate imbalance from continuous respiration in the dark, which is likely the reason for the reduced shoot biomass of the plants during the three-day transportation simulation. The addition of light can help maintain dry plant biomass and limit succulent stem elongation at above-optimal storage temperatures, but this is not always financially feasible during transit (Heins et al., 1992; Kubota and Kroggel, 2006).

The quality and morphology parameters of root biomass, leaf area, stem diameter, and the shoot:root ratio (graft only) were not sensitive to the stress conditions tested here. Also, the reductions in shoot biomass and plant compactness described above where not visually obvious symptoms. A more thorough understanding of what quality parameters are the most important to growers purchasing high-quality grafted seedlings would help determine the limit of marketability from transport-related symptoms and quality issues.

After one week of growth in the greenhouse, biomass from all exposure treatments was equivalent to the control plants that remained in the greenhouse, so early performance was not significantly impacted by transportation. The early growth rates of the plants and early flowering were also not impacted due to exposure treatments. It can be concluded that a critical

temperature that negatively affects post-transplanting growth is above 33 ± 0.6 °C. It should also be noted that transplanting into more extreme conditions such as open-field or high tunnels could also result in more stress or reduced survival than the greenhouse conditions tested here.

Conclusion

The results of this experiment indicate that grafted tomato seedlings (4-5 leaf stage) that are stored or transported under dark conditions will experience dose-specific physiological stress under moderate heat stress conditions, as measured by F_v/F_m. This stress is reversible after the plants are returned to normal light and growing temperatures. Dark transportation in above-optimal temperatures does have adverse effects on transplant quality. Grafted plants had reduced plant compactness and dry shoot biomass due to transportation temperatures at 23.8 ± 0.3 °C or above. Succulent stem elongation was a significant source of quality deterioration in the nongrafted plants assessed here. Our results indicate that grafted tomato plants may be better equipped to tolerate moderate heat stress during storage or transportation than nongrafted plants because they had a less severe decline in F_v/F_m and did not significantly elongate. However, reduced transplant quality did not have significant impacts on post-transplanting performance and early growth or flowering. It can be concluded grafted plants can tolerate temperatures up to 33 ± 0.6 °C for 48 hours during dark long-distance transportation without declines in post-transplanting performance. Therefore, it may be feasible to ship young grafted tomato plants (4-5 true leaves) without environmental control if external/outdoor temperatures are moderate. However, environmental control during the transportation is recommended to maintain grafted plant quality. The quality of the plants is likely an important factor for grafting nurseries selling higher-priced seedlings with the goal of customer satisfaction.

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Chapter 4- Effect of rootstock on 'Tasti-Lee' tomato yield and fruit quality in a high tunnel production system

Abstract

Grafting tomatoes with vigorous rootstocks can be used to increase yield in high tunnels without significant soilborne disease pressure. However, there is evidence suggesting that grafting with high-yielding rootstocks could compromise the accumulation of primary and secondary metabolites. 'Tasti Lee' is a hybrid tomato that is bred to have superior fresh-eating quality and higher lycopene content. The objective of this experiment was to investigate the yield and fruit quality impacts from grafting 'Tasti Lee' with rootstocks that range in their vigor and typical yield performance in high tunnels. Nongrafted 'Tasti-Lee' and 'Tasti-Lee' scion grafted onto 'Maxifort,' 'DRO141TX,' 'Fortamino,' 'Estamino,'and 'RST-04-106-T' rootstocks were trialed in a high tunnel in Kansas for three consecutive growing seasons (2018-20). The trials were arranged in a randomized complete block design with four replications. Total yield, marketable yield, average fruit size, and distribution of fruit size classes were assessed. Red ripe tomato fruit were harvested to determine: soluble solids content (SSC), titratable acidity (TA), and lycopene content, vitamin C content, antioxidant capacity (FRAP), and fruit firmness. 'Maxifort', 'DRO141TX', 'Estamino', and 'Fortamino' significantly increased marketable yields ($kg \cdot plant^{-1}$) by 31.5%-47% above nongrafted plants. In contrast, 'RST-04-106-T' did not lend any significant yield benefit. Regardless of rootstock, grafting increased the marketable average fruit weight by 20 g. Grafting did not cause significant effects in any of the fruit quality attributes assessed. However, the SSC of fruit from plants grafted to 'RST-04-106' was 10% higher ($P < 0.05$) than those grafted to 'Maxifort,' indicating that rootstock genotype can influence this

quality trait. Our findings suggest that growers can graft the tomato 'Tasti-Lee' with select vigorous rootstocks to increase marketable yields without sacrificing fruit quality for high tunnel production.

Introduction

Consumers are increasingly dissatisfied with the flavor of modern tomato cultivars and are willing to pay higher prices for better-flavored and/or locally-grown specialty tomatoes (Jordan, 2007; Tieman et al., 2017). In response, tomato breeding programs have focused more on breeding for improved flavor and nutrition (Baldwin et al., 2015). 'Tasti-Lee' is a determinate, fresh-eating cultivar that is professionally marketed by Bejo Seeds and sold as a premium tomato (Scott et al., 2008). The tomato has deep red pigment and up to 40% more lycopene content compared to other fresh-eating cultivars because it contains the crimson (og^c) gene (Scott et al., 2008). The cultivar is harvested vine-ripe and has improved flavor, a long shelf life, and high consumer acceptance (Cantliffe et al., 2009; Scott et al., 2008).

In the Central U.S., growers predominantly cultivate tomatoes in high tunnels. 'Tasti-Lee' could offer high tunnel growers a high-value and unique product for marketing to consumers, restaurants, and other regional markets. Vegetable grafting is an easily-adopted, compatible practice that can improve tomato crop performance in high tunnels. Previous high tunnel trials in Kansas have found that grafting 'Tasti-Lee' to the rootstock 'Maxifort' can increase marketable yields by 59.5% above nongrafted plants. Although vegetable grafting is predominantly used as an IPM tool to control soil-borne and foliar pathogens (Louws et al., 2010), the technology can improve tolerance to abiotic stress that is common in high tunnel cultivation. For instance, intensive production that relies on recurrent irrigation and fertilizer applications, along with a lack of rainfall, can increase soil salinity in high tunnels (Guan, 2016).

Temperatures may also become elevated in passively vented high tunnels during warm summer months (Wien, 2009). Air and soil temperatures may be too cool for optimal plant growth in the spring (Guan et al., 2018; Hunter et al., 2012). With the proper rootstock selection, grafted plants can provide tolerance from abiotic stressors such as high salinity (Fernández-Garcí et al., 2004; G. Colla et al., 2010), heat (Abdelmageed and Gruda, 2009; Rivero et al., 2003), chilling (Suchoff, et al., 2018), and water stress (Sánchez-Rodríguez et al., 2012)—such conditions that may arise in high tunnel cultivation. The use of vigorous rootstocks has often been found to increase marketable tomato yields in Central U.S. high tunnel cultivation where little to no disease pressure is evident (Lang et al., 2020; Loewen et al., in press; Masterson et al., 2016; Meyer et al., 2021). In addition to abiotic stress tolerance, improved growth and yield of grafted plants has been attributed to enhanced water-use efficiency (Djidonou et al., 2013; Suchoff et al., 2018) and nitrogen use efficiency (Albornoz et al., 2020; Djidonou et al., 2013) from vigorous root systems.

There are wide-ranging results concerning the fruit quality impacts from grafting in the literature. Some graft combinations have caused reductions in soluble solid content (SSC) (Casals et al., 2018; Mauro, et al., 2020b; Milenković et al., 2020; Turhan et al., 2011), vitamin C (Koleška et al., 2018; Nicoletto et al., 2013; Turhan et al., 2011), antioxidant concentrations (Moreno et al., 2019; Nicoletto et al., 2013; Riga et al., 2016) and increases in titratable acidity (Khah et al., 2006; Turhan et al., 2011). There are also scion x rootstock x environment interactions influencing fruit quality traits (Albacete et al., 2015; Cohen et al., 2007; Djidonou et al., 2020), which highlights the importance of conducting production-system specific rootstock trials for scions of interest.

Additionally, the degree of vigor afforded by the rootstocks may influence fruit compositional changes (Mauro et al., 2020a; Mauro et al., 2020b). For instance, antioxidant capacity was increased when the scion 'BHN 589' was grafted to RST-04-106-T rootstock that provided no yield benefit when utilized in high tunnels. Yet, the higher-yielding rootstock 'Maxifort' produced fruit with a comparable antioxidant capacity to nongrafted plants (Meyer et al., 2021). The rootstocks selected for this study were chosen because they range in their typical yield performance.

'Maxifort' is an interspecific rootstock classified as having high vegetative vigor, and it often improves yield in high tunnels with low disease pressure (Loewen et al., in press; Masterson et al., 2016; Meyer et al., 2021). 'DRO141TX' is also classified as a highly vigorous rootstock by the seed companies, but less has been reported in the literature. Similarly, 'Estamino' is described as having generative vigor by adding more energy to fruit production than vegetative production. Lang et al. (2020) found that 'DRO141TX' and 'Estamino' performed similarly to 'Maxifort' in high-tunnel tomato production with the scion 'BHN 589'. 'Fortamino' is even less-studied but is recommended for early vegetative vigor and increasing the number of flowers per truss and average fruit size. Preliminary data from Kansas has shown that it increases yield with 'BHN 589' scion (Rivard, unpublished data). 'RST-04-106-T' was included because it typically does not provide yield benefits in high tunnel production where little disease pressure is present (Lang and Nair, 2019; Meyer et al., 2021).

Because 'Tasti-Lee' is marketed specifically for its premium flavor and nutritional quality, and grafting could potentially compromise this attribute, it is critical to understand how grafting with vigorous rootstocks affects this particular variety of tomato. More specifically, the objectives of this study were to: (i) report the effect of grafting the scion 'Tasti-Lee' to high- and

low-yielding rootstocks for high tunnel production in regards to yield and fruit size; (ii) investigate the effect of these rootstocks on fruit quality; and (iii) determine if rootstocks impact flavor and nutritional quality parameters differently.

Materials and Methods

High tunnel trials were conducted in 2018, 2019, and 2020 at the Kansas State University's Olathe Horticulture Research and Extension Center (OHREC). The trials were arranged in a randomized complete block design with four replications and the arrangement of the plots were re-randomized each year. The treatments consisted of nongrafted 'Tasti-Lee' as well as five rootstock treatments with 'Tasti-Lee' scion grafted onto: 'Maxifort' (De Ruiter, St. Louis, MO), 'DRO-141-TX' (De Ruiter, St. Louis, MO), 'RST-04-106-T' (DP Seeds, Yuma, AZ), 'Fortamino,' (Enza Zaden, Salinas, CA) and 'Esatmino' (Enza Zaden, Salinas, CA) rootstocks. Fruit from one peak harvest in 2018 and 2019 and two harvests in 2020 were used for fruit quality analysis. All fruit quality evaluations were conducted in the Kansas State University Postharvest Physiology Lab at the KSU-Olathe Campus

Transplant production

The grafted and nongrafted plants were propagated in a greenhouse at the OHREC using the splice/tube grafting method outlined by Rivard and Louws (2011). The seeds of rootstocks and the scion were sown in a commercial germination mix and then transplanted into 50-cell propagation trays filled with Metro-Mix 852 Professional Growing Mix (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). At the two to four true leaf stage, the plants were splice grafted and joined using silicon clips (Hydro-Gardens, Colorado Springs, CO). The plants were then transferred to a healing chamber in the greenhouse that was covered with polyethylene film and 55% shade cloth. The chambers had cool-mist humidifiers to maintain high relative humidity.

The plants were removed from the healing chambers after 7 to 10 days and grown in the greenhouse for at least 10-14 more days before being transplanted into the high tunnel.

High tunnel trials

The high tunnel trial took place in a 9.1 m x 9.5 m moveable high tunnel (Rimol Greenhouse Systems; Hooksett, NH) at the Olathe Horticulture Center, located in Johnson County, KS (38.884347, -94.993426). The movable tunnel has three set positions on a 200' track and each year the high tunnel was in a different position. The soil type is chase silt loam. Four 16.5 m long beds running lengthwise with the tunnel and oriented north and south were the replications/blocks. Each treatment was randomly assigned to one of six plots in each bed. Each plot had five plants, and the in-row spacing was 18 inches. Standard high tunnel cultural practices were followed, including a raised bed plasticulture system with drip irrigation. Nutrients were supplied with a granular pre-plant fertilizer (31-16-16) at 22 $kg \cdot ha^{-1}$ and two fertigation events with potassium nitrate at 11.2 $kg \cdot ha^{-1}$ each. The between-row weeds were managed using fabric mulch, and the tomatoes were trained using a stake and weave vertical trellis system. The main pest pressure was from tomato fruitworms and hornworms. Once worms were observed, Bt was sprayed bi-weekly until the end of the growing season.

Harvesting and yield

Based on the USDA maturity standards, all fruit from the breaker stage to the red maturity stage were harvested each week (U.S. Standards for Grades of Fresh Tomatoes, 1991). In 2018, this occurred from July 2 to October 18. In 2019, fruit was harvested from July 26 to October 1, and from June 20 to October 16 in 2020. The fruit from each plot were separated by marketability, as determined by being free of large cracks, pest damage, rot, and blossom end rot. Marketable fruit were sorted according to the USDA size classes: small, medium, large, and

extra-large (U.S. Standards for Grades of Fresh Tomatoes, 1991). The various sizes of marketable fruit and the unmarketable fruit were counted and weighed. All fruit larger than 5 cm were harvested, counted, and weighed on the last harvest day of the season.

Fruit quality analysis

Fruit sampling for quality assessment. Tomatoes at the red maturity stage from each experimental unit were harvested for quality analysis. The harvest dates for the fruit quality analysis were: 18 Oct in 2018, 1 Oct in 2019, and 31 Aug and 8 Sept in 2020. On the day of harvest, the fruit was transported in an air-conditioned vehicle to the Postharvest Physiology Lab (approximately a 20-minute drive). To ensure the fruit were at a homogenous maturity stage, external color data were collected on each fruit by taking two measurements at opposite 45-degree angles on the blossom end with an A5 Chroma-Meter (Minolta CR-400; Minolta Co. Ltd., Osaka, Japan). All fruit had an average "a*" value of 25.4 ±1.7 and an a*/b* of 0.9 ± 0.8. The a* represents the degree of redness and b* is the degree of yellowness. The a*/b* ratio increases as tomatoes ripen it can be used as an indicator of the maturity stage (Batu, 2004; Brajovic et al., 2012)

Reagents and chemicals. All chemicals and reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The lycopene standard reference material was purchased from Millipore Sigma (Burlington, MA, USA).

Fruit firmness and organoleptic quality. In 2020 only, fruit firmness was measured on five tomatoes from each experimental unit using a TA-58, TA.XT.plus texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA). With a TA-30 75 mm diameter flat plate, the peak force needed for 2 mm compression deformation in grams (g) was measured. Firmness (N)

values were calculated as the average slope in the deformation curve divided by the peak force (g) as described by Jackman et al. (1990).

After firmness was measured, the fruit was cut into quarters from the stem end to the blossom end. One quarter from each of the five fruit per replication was blended, and 20 g of the puree was centrifuged. The hydrophilic supernatant was used for the determination of titratable acidity (TA) and SSC. Approximately 2 g of the blended tissue was homogenized with 20 mL of 6% metaphosphoric acid with 2N acetic acid solution and frozen at -20 °C until analysis of asorbic acid (AsA). The remaining portions of the tomatoes were frozen at -20 °C until analysis of lycopene and antioxidant capacity.

For TA, 5 mL of the tomato supernatant was mixed with 45mL of deionized water and measured with an automated titrator (862 Food/Beverage Compact titrosampler, Metrohm, Herisau, Switzerland). The results are reported as % citric acid equivalent. The samples' SSC was measured by dropping a drop of the hydrophilic tomato fraction on a hand-held digital refractometer (AR200, Reichert, Depew, NY, USA). The results are reported as °Brix.

AsA determination. Determination of AsA was based on the method by Klimczak and Gliszczynska-wiglo (2015). The previously frozen extracts were thawed, vortexed, and centrifuged. The supernatant was further diluted with 6% metaphosphoric acid/2N acetic acid solution (1 supernatant: 4 solution) and filtered through a 1 mL 96-well 0.22 μm filter plate (AcroPrep; Pall Co., Port Washington, NY) into a 2 mL 96-well plate (SKU: 186002482, Waters Co., Milford, MA, USA). Samples were read on a Waters Aquity ultra performance liquid chromatographer (UPLC) equipped with a photodiode array detector (PDA) and an Acquity BEH C18 column (Waters Co., Milford, MA, USA). Each well was injected in triplicate with 5 μL sample volume. The mobile phases consisted of 5 mM potassium phosphate

monobasic (KH_2PO_4), pH 2.65 with 0.1% of formic acid (mobile phase A), and methanol with 0.1% of formic acid (mobile phase B). At a flow rate of 0.2 mL/minute, the solvent management was a linear gradient starting with 5% A, with an increase to 15% A over 1 min, and then to 35% A over 1 min, and a return to the initial conditions over the next 4 min. An external five-point analytical AsA standard curve was made on the day of analysis with 10% meta-phosphoric acid solution and ranged from 2.5 to 5 $\mu g \cdot mL^{-1}$. The standard curve was used for the quantification of chromatograms read at 245nm. Ascorbic acid content is reported as mg AsA per100g fresh weight (FW).

Lycopene determination. The remaining frozen tomato samples were lyophilized in a freeze dryer prior to lycopene and antioxidant analysis (Harvest Right, Salt Lake City, Utah, USA). The extraction and UPLC/UV detection method for the determination of lycopene was based on a method by Maurer et al. (2014), with slight modification. The light was reduced during sample extraction and when handling standard reference material to avoid carotenoid degradation. 0.05 g of finely ground lyophilized tissue was weighed into 30mL polypropylene copolymer extraction tubes. 6mL of acetone/methanol (2:1, v/v; 0.5% butylated hydroxytoluene (BHT)) and 3 mL of hexane with 0.5% BHT were added to the tubes, vortexed, and sonicated in an ice bath for 20 minutes. To promote phase separation, 5 mL of 1 M sodium chloride solution was added. The sample extract was centrifuged at 1800 rpm for 10 minutes at 4 °C. An aliquot from the hexane layer was collected and filtered with 0.22 um, 13-mm PTFE syringe filters (VWR, Wayne, PA) into 2 mL amber sample vials (Waters, Milford MA) for analysis. Lycopene standard reference material was dissolved in hexane (0.5% BHT) and used for making an external standard curve ranging from 1.875 $ug \cdot mL^{-1}$ to 60 $ug \cdot mL^{-1}$ (Millipore Sigma, Burlington, MA).

A Waters Aquity UPLC, a reverse phased HSS C_{18} 2.1 x 100mm, 1.8 µm column, and a photodiode array detector (PDA) were used for sample analysis. The mobile phase A consisted of 75:23:2 acetonitrile/water/hexane (v/v/v, 0.1% acetic acid v/v). Mobile phase B was 90:80:2 acetonitrile/butanol/hexane (v/v/v, 0.1% acetic acid v/v). At a flow rate of 0.5 $mL \cdot min^{-1}$, the solvent gradient consisted of a 0.5 min hold on 100% A, a 1 min gradient to 80% B, a 0.1 min shift to 100% B, followed by 100% A until 13.5 min. Absorbance was recorded at 450, 280, and 350 nm. Samples were quantified with the lycopene standard curve and expressed as lycopene equivalent in $\mu g \cdot g^{-1}$ dry weight basis (D.W.).

Antioxidant capacity analysis (FRAP). The sample preparation and extraction of antioxidants was based on a method by Ou et al. (2002). Briefly, 0.25-g of lyophilized tissue were added to 10 mL of acetone/water (50:50, v/v) extraction solution. The solution was shaken at 400 rpm on an orbital shaker for one hour and then centrifuged (10,000 rpm, 20 min, 4 °C). The extract was further diluted by adding 1120 µl of acetone/water (50:50, v/v) to 80 µl of the extract. The antioxidant capacity was analyzed using the ferric reducing ability of plasma (FRAP) method (Benzie and Strain, 1996). 50 µl of the diluted sample extract was added to 180 µl FRAP reagent (ferric chloride/tripyridyltriazine (TPTZ)/ acetate buffer), and each sample was pipetted into a 96-well microplate in triplicate. Antioxidants in the sample extract reduce the ferric chloride/2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) reagent and change the solution color to blue. The plate was read with a Synergy H1 spectrophotometer at 593 nm (BioTek Winooski, VT). A 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) positive control curve ranging from 10 to 60 $\mu mol \cdot L^{-1}$ was used for quantification, and final results were expressed as µmol Trolox equivalents (TE) per g DW.

Statistical Analysis

All data analysis was performed with SAS (SAS version 9.4, Cary, NC, USA). The PROC GLIMMIX model with DDFM=KR in the MODEL statement was used for all analyses. The yield parameters were determined on a per plant basis for analysis and presentation of the data. For the yield-related response variables, the fixed effects of rootstock treatments, year, and a treatment x year interaction were modeled with block and treatment x block effects in the RANDOM statement. The least significant difference (LSD) was used for all mean separation. For the fruit quality data, the two harvests in 2020 were combined because there were no significant rootstock x harvest interactions. The postharvest data was analyzed as described for the yield data, but the SSC/TA ratio and lycopene content were natural log-transformed to improve data normality. All data were back-transformed for presentation. Tukey's HSD test was used for post-hoc mean separation for postharvest data.

Results

Yield

Table 4.1 provides the probability values of the effects of rootstock treatments and year on yield parameters. There was a significant effect of year on the total and marketable yield parameter, but no significant interactions between year and rootstock. Therefore, the three years were combined and the main effects of grafting were considered. All of the rootstocks except for 'RST-04-106-T' increased the total ($P \leq 0.01$) and marketable ($P \leq 0.05$) weight of fruit harvested compared to the nongrafted plants (Table 4.2). Marketable yields increased by 31.6% to 47.2% compared with the nongrafted from grafting to the other four rootstocks (Table 4.2). Grafting with 'RST-04-106-T' did not significantly increase marketable yield, but it was also not statistically lower than those achieved by 'Maxifort,' 'DRO141TX,' or 'Estamino.' The number

of marketable fruits was not significantly impacted by grafting, but the total number of fruits harvested was significantly increased when 'Tasti-Lee' was grafted to 'Maxifort,' 'Fortamino' and 'Estamino' ($P \leq 0.01$).

Regardless of rootstock, grafting increased the average weight of marketable ($P \leq 0.01$) and total fruit ($P \leq 0.001$). The marketable fruit from grafted plants was, on average, 20g heavier than fruit from nongrafted 'Tasti-Lee.' The number of extra-large fruits harvested was significantly increase from grafting with all rootstocks except for 'RST-04-106-T' ($P \leq 0.01$; Table 4.3) and plants grafted with 'Maxifort' produced more than twice as many extra-large fruits as the nongrafted plants.

Table 4.1 Probability values of yield parameters from overall ANOVA F-Test of main effects: grafting treatments and production year.

	Treatment[z]	Year[y]	Treatement x Year
Marketable weight (kg·plant^{-1})	0.0275	<.0001	ns[x]
Marketable No./plant	0.1359	<.0001	ns
Average marketable fruit weight(g)	0.0022	<.0001	ns
Total weight (kg·plant^{-1})	0.0041	<.0001	ns
Total No./ plant	0.0093	<.0001	ns
Average total fruit weight (g)	0.0006	<.0001	ns
Small fruit (No./plant)	ns	ns	ns
Medium fruit (No./plant)	ns	ns	ns
Large fruit (No./plant)	ns	<.0001	ns
Jumbo fruit (No./plant)	0.0003	<.0001	ns

[z]Treatment consisted of 5 rootstocks grafted to 'Tasti-Lee' and nongrafted controls.
[y]Year is the effect of growing season: 2018, 2019, and 2020.
[x]Ns, non-significant at $\alpha=0.05$

Table 4.2 Tomato fruit yield of nongrafted and grafted 'Tasti-Lee' tomatoes grown in a high tunnel at the Olathe Horticulture Center in 2018, 2019, and 2020.

	Marketable Yield[z]			Total Yield		
Treatment[y]	Weight (kg·plant-1)	No./plant	Average fruit weight (g)	Weight (kg·plant-1)	No./plant	Average fruit weight (g)
Nongrafted	2.69 c	16.4	165.6 b	4.21 c	26.6 c	159.6 c
Maxifort	3.61 ab	19.1	188.7 a	5.96 ab	33.2 ab	180.2 ab
DRO141TX	3.54 ab	18.4	190.1 a	5.65 ab	31.1 abc	181.1 a
Fortamino	3.96 a	21.5	183.3 a	6.33 a	36.2a	173.5 ab
Estamino	3.81 ab	20.5	186.1 a	5.94 ab	34.0 ab	175.5 ab
RST04106	3.04 bc	16.6	180.8 a	4.88 bc	28.4 bc	170.4 b
F-test[x]	0.028	ns	0.002	0.0041	0.0093	0.001

[z]Fruit was harvested weekly in 2018, 2019, 2020 and marketability was determined by fruit free from large cracks, pest damage, rot, and blossom end rot. Total and marketable fruit were counted and weighed each week and values are presented on a per plant basis.
[y]Nongrafted treatment was 'Tasti-Lee' and the other treatments are the given rootstock grafted with 'Tasti-Lee' as the scion.
Means followed by the same letter are not significantly different according to LSD paired comparisons where α=0.05
[x]Probability value for the overall ANOVA F-test using Type III hypothesis test where α=0.05

Table 4.3 Tomato size distribution of marketable fruit harvest from grafted and nongrafted 'Tasti-Lee' tomato grown in a high tunnel at the Olathe Horticulture Center in 2018, 2019, and 2020

Treatment[y]	Small (No./plant) [z]	% Small	Med. (No./plant)	% Med.	Large (No./plant)	% Lg.	Extra-large (No./plant)	Extra-large (%)
Nongrafted	0.63	3.6	3.50	22.9	9.26	55.6	3.0 c	17.8
Maxifort	0.34	1.9	2.68	14.2	9.49	50.4	6.6 a	33.6
DRO141TX	0.42	2.2	2.95	16.7	9.13	48.7	5.9 ab	32.4
Fortamino	0.59	2.6	4.25	19.7	10.95	51.3	5.7 ab	26.5
Estamino	0.61	2.7	3.72	17.5	10.34	51.3	5.9 ab	28.5
RST-04-106-T	0.50	3.1	3.40	21.0	8.27	49.3	4.4 bc	26.6
F-test[x]	ns		ns		ns		0.0003	

[z]Fruit size class number and class percentage of total fruit number of marketable fruit harvested weekly in 2018, 2019, and 2020.

[y]Nongrafted treatment was 'Tasti-Lee' and the other treatments are the given rootstock grafted with 'Tasti-Lee' as the scion.
Means followed by the same letter are not significantly different according to LSD paired comparisons where α=0.05
[x]Probability value for the overall ANOVA F-test using Type III hypothesis test where α=0.05.

Fruit quality

The probability values of the main effects of grafting/rootstock and year for fruit quality parameters can be found in Table 4.4. There were no significant interactions between year and rootstock, so only the effect of rootstocks is considered. There was significant year-to-year variation for all fruit quality criterium assessed except for antioxidant capacity (FRAP) (Table 4.4). Rootstock treatments only led to significant effects in SSC.

The SSC of 'Tasti-Lee' tomatoes from all treatments ranged from 4.31°Brix to 4.67 °Brix (Table 4.5). The tomatoes grafted to 'RST-04-106-T' had the highest SSC (4.67 °Brix) and were significantly greater than those grafted to 'Maxifort,' which had the lowest SSC (4.31°Brix) ($P \leq 0.05$). All of the rootstock treatments had statistically similar SSC to the nongrafted 'Tasti-Lee' tomatoes. There were no significant differences in the TA or the SSC/TA of fruit from any rootstock treatment.

The antioxidant capacity (FRAP), lycopene content, and ascorbic acid content were statistically similar across all treatments (Table 4.6). Numerically, the rootstock 'RST-04-106-T' had the highest mean lycopene content of 842.55 $\mu g \cdot g^{-1}$ DW, and nongrafted plants had the lowest at 534.81 $\mu g \cdot g^{-1}$ DW. Fruit firmness was only assessed in 2020 across two harvests. There was no significant effect of harvest days, so the treatment means across both harvests are provided in Figure 4.1. There were no significant treatment effects on fruit firmness (N/mm), and the average firmness value was 19.1 ± 4.7 N/mm.

Table 4.4 Probability value of postharvest quality parameters from overall ANOVA F-Test of main effects: grafting treatments and production year.

	Treatment[z]	Year[y]	Treatment x Year
SSC	0.0167	0.0025	ns
TA	ns	0.0037	ns
SSC/TA	ns	0.0382	ns
Lycopene	ns	<.0001	ns
Antioxidant capacity (FRAP)	ns	ns	ns
Ascorbic Acid	ns	0.0003	ns

[z]Treatment consisted of 5 rootstocks grafted to 'Tasti-Lee' and nongrafted controls.
[y]Year is the effect of growing season: 2018, 2019, and 2020.

Table 4.5 Organoleptic fruit quality soluble solids content (SSC), titratable acidity (%TA) and the SSC/%TA ratio of 'Tasti-Lee' tomatoes harvested during 2018, 2019, and 2020.

Treatment[z]	SSC	TA (% citric acid)	SSC/TA
Nongrafted	4.53 ab[y]	0.311	14.6
Maxifort	4.31 b	0.320	13.6
DRO141TX	4.38 ab	0.316	14.5
Fortamino	4.37 ab	0.301	15.6
Estamino	4.43 ab	0.313	14.2
RST04106T	4.67 a	0.300	15.3
F-test[x]	0.0167	ns	ns

[z]Treatments consist of nongrafted 'Tasti-Lee' and the given rootstock grafted to 'Tasti-Lee'.
[y]Values represent the LSMEANS of fruit harvested at the red ripeness stage from one harvest in 2018 and 2019 and two harvests in 2020.
LSMEANS followed by the same letters are not significantly different (P>0.05) according to pairwise comparisons with a Tukey's adjustment.
[x]Probability value for the overall ANOVA F-test using Type III hypothesis test where α=0.05.

Table 4.6 Nutritional fruit quality: lycopene, FRAP, and ascorbic acid content of 'Tasti-Lee' tomatoes harvested during 2018, 2019, and 2020.

Treatment[z]	Lycopene (µg·g–1 DW)	FRAP (TE/100 g DW)	Ascorbic acid (mg /100g FW)
Nongrafted	493.09[y]	514.52	24.96
Maxifort	579.52	463.18	21.75
DRO141TX	524.53	459.91	23.93
Fortamino	472.295	486.17	21.13
Estamino	543.92	457.94	23.23
RST-04-106-T	655.83	458.95	22.82
F-test[x]	ns	ns	ns

[z]Treatments consist of nongrafted 'Tasti-Lee' and the given rootstock grafted to 'Tasti-Lee'.
[y]Values represent the LSMEANS of fruit harvested at the red ripeness stage from one harvest in 2018 and 2019 and two harvests in 2020.
LSMEANS followed by the same letters are not significantly different (P>0.05) according to pairwise comparisons with a Tukey's adjustment.
[x]Probability value for the overall ANOVA F-test using Type III hypothesis test where α=0.05.

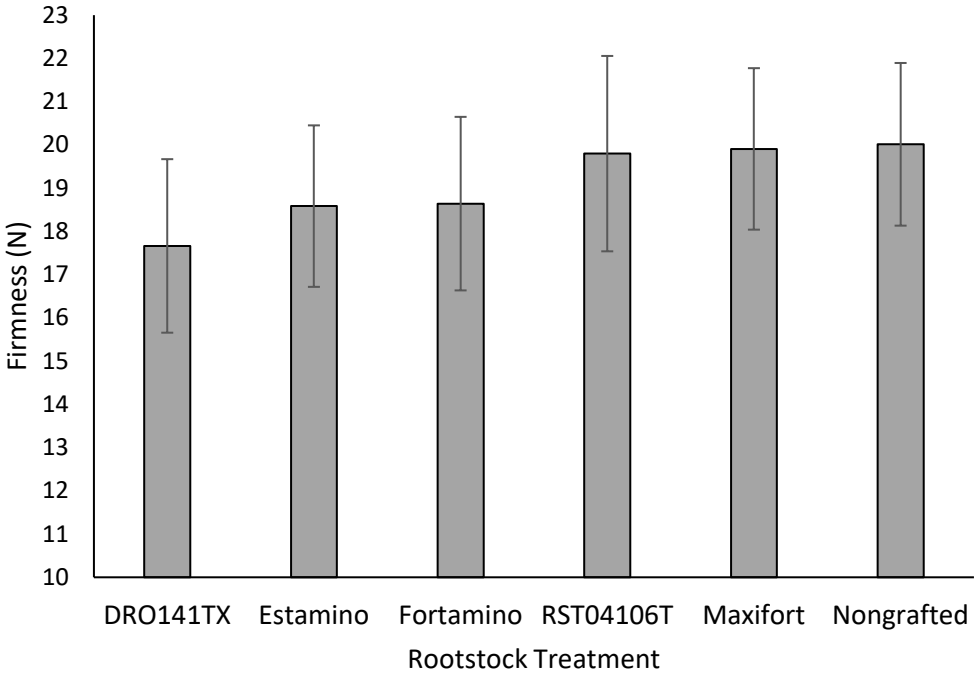

Figure 4.1 Average fruit firmness of fruit harvested at the red maturity stage 2020. Bars represent LSMEANS for each rootstock treatment group and error bars are the standard error of the mean. Firmness was measured with a TA-58, TA.XT.plus texture analyzer a using a flat-plate

compression method. There were no significant differences in treatments according to an overall F-test using Type III hypothesis test where $\alpha=0.05$.

Discussion

This trial aimed to understand the effects of different rootstocks on the yield and fruit quality of the cultivar 'Tasti-Lee' in a high tunnel production system. The overall yields achieved in this trial were lower than previously reported for grafted and nongrafted 'Tasti-Lee' in high tunnel trials conducted in a different high tunnel structure at the same location (Oxley et al., 2015; Loewen et al., in press). Loewen et al. (in press) found that marketable yields for nongrafted 'Tasti-Lee' plants ranged from 5.7-6.7 $kg \cdot plant^{-1}$, while the average in this trial was 2.69 $kg \cdot plant^{-1}$. Loewen et al. (in press) also reported that marketable yield increased by 59.5% when grafted to 'Maxifort,' while the increase was 34.2% in this trial. Although cultivation practices were similar, the trials took place within a different high tunnel structure and experienced varied environmental and soil conditions and pest pressure, which could result in the discrepancy. . Additionally, the plants were transplanted into the high tunnels an average of 45 days later compared to the previously mentioned trials, which likely accounts for reduced overall yields. Later planting dates can also result in higher temperatures during initial flowering, which interfere with proper pollination and reduced yields (Rogers and Wszelaki, 2012).

There was a significant year-to-year variation in yield, but the rootstocks performed similarly relative to each year. The rootstocks 'Maxifort,' 'DRO141TX', 'Fortamino,' and 'Estamino' increased marketable yield compared to the nongrafted 'Tasti-Lee' plants. 'RST-04-106-T' did not significantly increase marketable yield, which has been reported with other scions grafted to this rootstock and grown in high tunnels without significant disease pressure (Meyer et

al., 2021; Lang et al., 2019, 2020).‘Maxifort’ is a popular rootstock in the U.S. and has often been found to increase marketable yields in protected culture systems without significant pathogen pressure (Djidonou et al., 2017; Lang et al., 2020; Masterson et al., 2016; Myer et al., 2020). Similar to the findings here, Lang et al. (2020) observed that ‘Estamino’ and ‘DRO141TX’ performed similarly to ‘Maxifort’ and increased marketable yields in high tunnel cultivation with the scion ‘BHN 589.’ ‘Fortamino’ has been less studied, but it achieved the highest marketable yield increases (47.2%) in this trial, although they were not significantly different than ‘Maxifort.’ This same graft combination (‘Tasti-Lee’/’Fortamino’) was also recently trialed in open-field production without disease pressure in North Carolina, where it increased marketable yields by 22% and performed similarly to the rootstock, ‘Beaufort.’ (Ingram, 2020).

‘Tasti-Lee’ typically produces smaller tomatoes compared to other beefsteak tomato cultivars, and increasing the average fruit size could also be desired from a marketing perspective. In our trials, we measured average fruit weight as an indicator of size and also sorted marketable fruit into USDA size grades. An increase in average fruit weight and size is one of the most consistent effects of vigorous, interspecific rootstocks (Kyriacou et al., 2017). In our investigation, all the rootstocks increased the average fruit weight of ’Tasti-Lee’ tomatoes by 9 to 15%. The number of extra-large fruit was also increased from all the rootstock other than ‘RST-04-106-T’. This degree of fruit weight increase is consistent with results from other vigorous rootstocks/scion combinations in open-field production (Djidonou et al., 2016; 2013), high tunnel production (Lang et al., 2020; Meyer et al. 2021; Masterson et al., 2016) and greenhouse production (Mauro et al., 2020b; Rahmatian et al., 2014). Loewen et al. (in press) also found that grafting ‘Tasti-Lee’ to the rootstock ‘Maxifort’ increased the average fruit weight

by an average of 33% in a similar high tunnel system but this effect was not significant. Ingram (2020) reported that 'Tasti-Lee' grafted to the rootstock 'Beaurfort' also increased the number of extra-large fruit harvested in open-field production. Contrary to our findings, the rootstock 'RST-04-106-T' did not act to increase the average fruit weight of the scion 'BHN 589' grown in a high tunnel (Lang et al., 2020; Meyer et al., 2021). This could be because the nongrafted 'Tasti-Lee' tomatoes were, on average, smaller (165.6 g) than nongrafted 'BHN 589', which can range from 173 g to 218 g (Lang et al., 2020; Meyer et al., 2021). The rootstock effects on fruit size and weight can also vary among rootstock/scion combinations (Turhan et al., 2011), from year to year (Lang et al., 2020; Masterson et al., 2016), and among different cultivation systems (Casals et al., 2018). A possible mechanism of rootstock-induced increases in fruit size could be related to an increased flow of assimilates and water during the fruit expansion phase. In general, increases in yield—either through increased fruit number and/or increased fruit size—of grafted plants grown without disease pressure are attributed to inherent vigor and improved nutrient and water uptake by vigorous root systems (Djidonou et al., 2013).

The SSC and TA found in this experiment are lower than previously reported from open-field production of 'Tasti-Lee' (Ingram, 2020; Scott et al., 2008) and higher than winter-grown greenhouse tomatoes (Cantliffe et al., 2009). However, the SSC/TA, ratio of the nongrafted tomatoes in this experiment agrees with those assessed over multiple seasons in Florida (Scott et al., 2008).

In this trial, grafting did not cause significant differences in the SSC, TA, or SSC/TA ratio of the fruit compared to nongrafted controls. But, the rootstock 'RST-04-106-T' did produce fruit with higher SSC than fruit harvested from plants grafted to 'Maxifort,' indicating that rootstock genotypes differently affected this quality trait. These organoleptic parameters are

essential for tomato taste and consumer acceptance (Causse et al., 2010). Therefore, our data suggests that the taste was not significantly impacted due to grafting. Considering that 'Tasti-Lee' is marketed for improved flavor, changes in these organoleptic parameters are important to understand. Future research should include consumer sensory analysis to investigate any perceived flavor or preference changes from grafting 'Tasti-Lee' or other improved-flavor scions.

The grafting effects on SSC and TA differ from Ingram (2020), who found that grafting 'Tasti-Lee' to 'Beaufort,' 'Arnold' and 'Shield' significantly reduced fruit SSC by 4 to 8% and grafting to 'Beaufort' and 'Shield' significantly reduced TA of the fruit. However, the numerical reductions in SSC from grafting to 'Maxifort' 'Fortamino' and 'DRO141TX' in this trial were similar and ranged from 3 to 5%. Vigorous rootstocks have often been reported to decrease SSC by an average of 9 to 13% in a variety of production systems (Al-Harbi et al., 2017; Casals et al., 2018; Mauro et al., 2020b; Milenković et al., 2020; Moreno et al., 2019; Pogonyi et al., 2005; Turhan et al., 2011). Increases in the TA of tomatoes have also been attributed to grafting with vigorous rootstocks such as 'Maxifort' (Krumbein and Schwarz, 2013; Meyer et al., 2021). Others have found no effect on fruit TA when grafting with this same rootstock (Djidonou et al., 2017; Lang et al., 2020)—similar to the findings here. These results suggest that TA is highly subject to rootstock x scion interactions.

The mechanisms of fruit composition effects from vigorous rootstocks are not clearly understood, but it is possible that the increased vegetative and root biomass act as additional assimilate sinks in competition with the fruit (Martínez-Ballesta et al., 2010; Mauro et al., 2020a; Mauro et al., 2020b). Improved water uptake from vigorous rootstocks may result in an increased flow of water to the fruit during fruit expansion, which could increase fruit size and dilute the

SSC (Mauro et al., 2020b;. Turhan et al., 2011). Increases in fruit load may also reduce assimilate partitioning into individual fruits. Interestingly, plants grafted to 'RST-04-106-T' produced mature-red fruit with the highest SSC among all treatments and nongrafted plants and was significantly higher than fruit from plants grafted to 'Maxifort.' 'RST-04-106-T' was the only rootstock in the trial to not significantly to increase yields and had fruit numbers closer to those of the nongrafted plants. Lang et al. (2020) also reported that 'RST-04-106-T' significantly increased SSC of 'BHN 589' above higher-yielding vigorous rootstocks, including 'Maxifort', 'Estamino, and 'DRO141TX' in one year of the trial. These results support the hypothesis that grafting-induced changes in SSC could be related to the overall vegetative vigor and/or fruit load of the grafted plants. Alternatively, changes could be due to direct changes in transcriptional processes of sugar metabolism from this rootstock. Watermelon grafted to bottle gourd rootstocks have been found to have differently expressed genes related to sugar metabolism and sugar transport (Aslam et al., 2020). Yet, the organoleptic quality of tomatoes is often subject to environmental variation (Panthee et al., 2012) and changes attributed to harvest period (Di Gioia et al., 2010; Djidonou et al., 2017) or year (Barrett et al., 2012; Lang et al., 2020) are often more significant than rootstock effects. It is important to note that the fruit in this study was only sampled one or two times in each year towards the end of the season when there was sufficient red fruit. Future investigations would benefit from more sampling periods and/or larger plots to confirm our results on the rootstocks effects on flavor quality of 'Tasti-Lee' tomatoes.

Grafting did not significantly affect the lycopene content, AsA content, and antioxidant capacity of 'Tasti-Lee' tomatoes, and seasonal variations led to greater concentration differences in these nutritional parameters. There were no significant variations in these functional compounds between the different rootstock assessed, so no conclusions can be made about how

rootstock vigor and yield performance may influence fruit nutritional compounds. Grafting-induced changes in functional compounds are often highly dependent on rootstock/scion combination, as well as interactions with the environment. Vigorous rootstocks have not affected lycopene (Khah et al., 2006; Djiondou et al., 2017, 2016) and have also decreased lycopene content (Gajc-Wolska et al., 2014; Helyes et al., 2009; Krumbein and Schwarz, 2013). In open-field production, grafting 'Tasti-Lee' to the rootstocks 'Beaufort' and 'Shield' significantly reduced lycopene by 6%, while the rootstock 'Arnold' was comparable to nongrafted plants (Ingram, 2020). The numerical differences in lycopene content between treatments were greater in this trial but not statistically significant, which likely speaks to greater variability within each rootstock treatment found in this trial in comparison to Ingram (2020). Yet, all the rootstocks trialed had, on average, greater lycopene content than the nongrafted plants, so we can suggest that grafting did not compromise the lycopene content of this high-lycopene cultivar.

Grafting to any of the rootstocks also did not affect antioxidant capacity of the fruit as measured by FRAP. Previously, the lower-yielding 'RST-04-106-T' rootstock was found to significantly increase FRAP over two years with the scion 'BHN-589' grown in high tunnel cultivation (Meyer et al., 2019). However, we did not observe any benefit or reduction in antioxidant capacity with 'Tasti-Lee' as the scion. Reductions in AsA are common when grafting to vigorous, interspecific rootstocks such as 'Maxifort' (Di Gioia et al., 2010; Ilić et al., 2020) and 'Beaufort' (Turhan et al., 2011), but the use of vigorous rootstock did not limit the accumulation of AsA in this trial. Lastly, grafting did not significantly impact the firmness of 'Tasti-Lee' tomatoes, which is important because firmness is a trademark characteristic of this cultivar (Scott et al., 2008).

Conclusion

As grafting technology is deployed in high tunnel and open-field systems across the U.S., growers need research-based information to determine if this strategy will work not only for their production systems, but also their customers. ‘Tasti-Lee’ is a marketed as a tomato with good flavor and nutritional quality, which could be affected by the use of vigorous rootstocks. The results of our trials suggest that grafting with select rootstocks increased the marketable yield of ‘Tasti-Lee’ tomatoes grown in a high tunnel without compromising quality. The SSC of tomatoes from the plants grafted to the ‘RST-04-106-T’ rootstock was significantly greater than those grafted to ‘Maxifort,’ suggesting rootstocks can differently influence fruit composition of this scion. Considering ‘Tasti-Lee’ can be marketed at a premium price, an economic analysis of a high tunnel grafting system with this scion would be useful for growers to make informed decisions as they implement integrated technologies such as vigorous rootstocks and high-lycopene scions.

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Chapter 5-Effect of rootstocks on carotenoid composition of mature red 'Tasti-Lee' tomatoes

Abstract

Tomatoes (*Solanum lycopersicum* L.) are considered a functional food due their high levels of carotenoids and other phytonutrients. Breeding efforts have been made to increase antioxidant lycopene content in tomatoes to improve the human health benefits. 'Tasti-Lee' is one such cultivar that is marketed for its increased lycopene content and improved flavor quality. This cultivar could be suitable for high tunnel cultivation, where grafted plants are often used to improve production quality. Yet, certain rootstock/scion combinations have been found to alter the accumulation of tomato carotenoids. The objective of this research was to assess the carotenoid response of this high-lycopene cultivar when grafted to five different rootstocks. 'Tasti Lee' tomatoes were grafted to 'Maxifort,' 'DRO 141 TX,' 'Fortamino,' 'Estamino,' and 'RST-04-106-T' rootstocks and grown in a high tunnel system for three years. The experiment was arranged in a randomized complete block design. Mature red fruit were harvested and used for carotenoid analysis. The carotenoids were detected and quantified using UPLC/UV. The most predominant carotenoid was lycopene, followed by β-carotene and phytofluene. The rootstocks assessed did not cause significant concentration differences compared to nongrafted plants for the three carotenoids identified. However, compositional data analysis showed that the ratio of β-carotene to the other two carotenoids was reduced when the rootstock 'RST-04-106-T' was used compared to the nongrafted plants. The composition of carotenoids did not differ in the other rootstocks assessed. Our results indicate that grafting 'Tasti-Lee' to select vigorous rootstock does not compromise carotenoid content or composition of this high-lycopene cultivar.

Introduction

Carotenoids are lipophilic molecules responsible for yellow, orange and red pigments in many vegetables, fruits, and flowers (Del Giudice et al., 2017). Tomatoes (*Solanum lycopersicum* L.) are unique because the fruit accumulates high amounts of the carotenoid lycopene in favor of β-carotene (Bramley, 2002).

The accumulation of carotenoids during tomato fruit ripening is regulated by enzymes and proteins under transcriptional control (Fraser et al., 2009). The isoprenoid precursor to all carotenoids is geranylgeranyl pyrophosphate (GGPP). Phytoene synthase (PSY) converts GGPP to phytoene, which is the first colorless carotenoid in the biosynthesis pathway. Phytoene is desaturated by phytoene desaturase (PDS) to form another colorless carotenoid: phtyofluene (Fraser et al., 2009). A series of desaturation steps extends the number of conjugated double bonds in each carotenoid in the pathway, which lends the compounds their characteristic pigments. All-*trans*-lycopene is the predominant lycopene isomer found in fresh tomatoes (Fraser et al., 2009). Lycopene has eleven conjugated double bonds, which provides the red color in tomato fruit. Either lycopene β-cyclase or lycopene ϵ-cyclase cyclizes lycopene to form β-carotene and δ-carotene, respectively (Bramley et al., 2002).

Epidemiological investigations have revealed that consumption of tomatoes and tomato-based products has human health benefits, such as the reduced risk of cardiovascular disease (Cheng et al., 2017) and prostate cancer (Giovannucci, 2005). The health-promoting benefits of tomato consumption have been attributed to the antioxidant function of lycopene and its more bioactive products (Erdman Jr et al., 2009; Fraser et al., 2009). Due to the widespread consumption of tomato products, tomatoes are one of the main sources of lycopene in the human diet (Canene-Adams et al., 2005). Yet, other carotenoids and phytochemicals in tomatoes are

likely to act in synergy with lycopene to provide human health benefits (Basu & Imrhan, 2007). β-carotene is the only tomato carotenoid that is a precursor to vitamin A, which is essential in the human diet and important for eye health (Tang, 2010). The minor carotenoid lutein is also found in tomatoes and can is associated with reduced risk of macular degeneration (Buscemi et al., 2018). Phytoene and phytofluene are also common dietary carotenoids readily found in tomatoes (Biehler et al., 2012). Although found in lower concentrations than lycopene, these carotenoids have been found to have similar health benefits as lycopene, and phytofluene may be more readily absorbed by humans than lycopene (Meléndez-Martínez et al., 2018; Richelle et al., 2002). Additionally, carotenoids are precursors to several aroma volatiles associated with flavor preference in tomatoes (Vogel et al., 2010). Therefore, the investigation of the carotenoids it is important for both flavor and nutritional properties of the tomato fruit.

'Tasti-Lee' is a fresh-eating tomato hybrid that is marketed to consumers for improved flavor and lycopene content—which is up to 40% higher than other fresh-eating tomatoes (Scott et al., 2008). This cultivar contains the crimson gene (og^c), which is a defect in lycopene β-cyclase that causes an increased accumulation of lycopene and reduced β-carotene content (Siddiqui et al., 2014). The greater lycopene content from this mutation may also be due to increased PSY activity (Siddiqui et al., 2014). This cultivar is primarily cultivated in open-field production in the southeastern United States. It is professionally marketed by Bejo Seeds and sold as a premium tomato in grocery stores (Scott et al., 2008).

In the central U.S., tomatoes are commonly cultivated in high tunnels and sold in local markets. Considering the market success of 'Tasti-Lee' in other parts of the country, this cultivar could offer high tunnel growers a unique cultivar that can be marketed to consumers for improved fruit quality characteristics. Tomato grafting is a compatible practice that can be used

to improve tomato crop performance in high tunnels (Lang et al., 2020.; Masterson et al., 2016; Meyer et al., 2021). Previous high-tunnel trials in Kansas have shown that grafting 'Tasti-Lee' to the vigorous rootstocks can increase marketable yields by 34.2% to 59.5% (Jenkins, 2021, Chapter 4; Loewen, 2018). Therefore, this cultivar could also produce comparable yields to other commonly-grown high tunnel cultivars when grafting is used.

The content of tomato carotenoids is predominantly under genetic control; however, there is evidence that carotenoid content can be influenced by grafting. For instance, Helyes et al. (2009) found that grafting two different scions to the rootstock 'Beaufort' reduced lycopene content by an average of 23.5% over three years of greenhouse cultivation. Often, grafting-induced reductions in lycopene content are small and only found in some of the rootstock/scion combinations assessed (Krumbein & Schwarz, 2013; Nicoletto et al., 2013; Schwarz et al., 2013). Schwarz et al. (2013) found that 'Maxifort' reduced lycopene content, but not β-carotene in the scion 'Classy'. Yet, this same rootstock was able to recover lycopene and β-carotene reductions when the plants were grown under a low potassium supply (Schwarz et al., 2013). There are also reports of some rootstock/scion combinations increasing lycopene content under non-stressed conditions (Fernández-García et al., 2004; Riga et al., 2016). Most investigations on the rootstock effect on tomato carotenoids only assess the major carotenoids of lycopene and β-carotene. However, Brajovic et al. (2012) found that rootstocks could influence the accumulation pattern of the minor carotenoid, lutein, during fruit ripening. These findings warrant further investigation into possible rootstock effects on the carotenoid composition.

To gain a better understanding of the relative carotenoid compositions in mature red tomatoes, we used compositional data analysis techniques similar to Brajovic et al. (2012). The analysis of compositional data requires special statistical techniques as described by Aitchison,

(1982) because each part of a composition is constrained by a fixed total. This compositional sample space is referred to as a simplex (Greenacre, 2018). Using data transformation techniques—such as logratios—moves the data from the simplex to a normal, unconstrained space that allows for standard statistical tests and inferences (Greenacre et al., 2021). Also, analysis of logratios between the parts is preferred over the analysis of individual parts because the ratios are said to have subcompositional coherence (Greenacre et al., 2018). In other words, the ratio between parts does not change if you are looking at sub-parts i.e. subcompositions, or the full composition (Greenacre et al., 2018). Whereas, summary statistics on individual parts in a composition will change when you add or subtract parts from the whole composition.

The influence on tomato carotenoids from grafting is especially important to characterize for scions marketed for improved flavor and lycopene such as 'Tasti-Lee'. The objectives of this investigation were to: (i) identify the effects on the concentrations of major and minor carotenoids of 'Tasti-Lee' tomatoes grafted to five different commercially available rootstocks; (ii) to determine the rootstock effects on the relative compositions of carotenoids using compositional data analysis techniques. This information will provide insight into possible rootstock/scion combination effects on the carotenoid composition, as well as provide growers with helpful information for making informed rootstock selections.

Materials and Methods

Transplant production.

The grafted and nongrafted tomato transplants were propagated in a Quonset-style greenhouse at the Olathe Horticulture Research and Extension Center. The determinate 'Tasti-Lee' was used as the scion and nongrafted control plants. The rootstocks evaluated were 'Maxifort' (De Ruiter, St. Louis, MO), 'DRO-141-TX' (De Ruiter, St. Louis, MO), 'RST-04-

106-T' (DP Seeds, Yuma, AZ), 'Fortamino,' (Enza Zaden, Salinas, CA) and 'Esatmino' (Enza Zaden, Salinas, CA). The greenhouse was maintained at 28 °C (day) to 18 °C (night). The seeds of rootstocks and the scion were sown in Fafard Germinating Mix in 30 cm x 30 cm flat trays seedling flats and then transplanted into 50-cell propagation trays filled with Metro-Mix 852 Professional Growing Mix (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). For the nongrafted 'Tasti-Lee' plants, we sowed the seeds approximately 7 to 10 days after the seeds for the grafted plants so the transplants would be at similar stages at the time of transplanting. We followed the grafting and healing protocol by Rivard and Louws (2011). When the rootstocks and scions had similar stem diameters and 3 to 4 true leaves, they were splice grafted and joined using silicon clips. After grafting, the plants were placed in healing chambers located in the greenhouse. The chambers were covered with polyethylene film and 55% shade cloth and contained a cool-mist humidifier. The plants remained in the healing chamber for 7 to 10 and were then grown in the original greenhouse environment for at least 10 to 14 more days before being transplanted into the high tunnel.

High tunnel trial

The grafted and nongrafted tomato plants were transplanted into a 9.1 m x 9.5 m moveable high tunnel (Rimol Greenhouse Systems; Hooksett, NH) located at the Olathe Horticulture Research and Extension Center (38.884347, -94.993426). The soil type at this location is chase silt loam. The experiment was arranged in a randomized complete block design with four replications and six treatments (five rootstocks and the nongrafted control). Four 16.5 m beds running lengthwise in the tunnel were the blocks and each bed contained six treatment plots (experimental unit). The treatments were randomly assigned to a plot within each row, and each plot contained five plants with an in-row spacing of 45.72 cm.

We managed the trial using standard high tunnel cultivation practices, including a raised bed plasticulture system with drip irrigation. A granular pre-plant fertilizer (31-16-16) was applied at a rate of 22 $kg \cdot ha^{-1,}$ and two fertigation applications with potassium nitrate were supplied at 11.2 $kg \cdot ha^{-1}$ each. The between-row weeds were suppressed using landscape fabric, and the plants were trained with a stake and weave vertical trellis system. The main pest pressure was from tomato fruitworms and hornworms, which were controlled with bi-weekly applications of Bt. The trials were conducted for three years—2018, 2019, and 2020. The tomato plants were transplanted on May 25 in 2018, on June 24 in 2019, and on June 5 in 2020.

Tomato sampling

For analysis of carotenoids, tomatoes were harvested at the red maturity stage according to the USDA tomato maturity standards (U.S. Standards for Grades of Fresh Tomatoes, 1991). Tomatoes from one peak harvest in 2018 and 2019 and two harvests in 2020 were transported to the Kansas State University Postharvest Physiology Lab in Olathe, KS in an air-conditioned vehicle for quality analysis. The harvest dates were as follows: October 18, 2018, October 1, 2019, August 31, 2020, and September 8, 2020. To ensure the homogeneity of the maturity stage, objective color measurements were collected on all harvested red fruit from each experimental unit. An A5 Chroma-Meter was used to take two color measurements at opposite 45-degree angles on the blossom end (Minolta CR-400; Minolta Co. Ltd., Osaka, Japan). If there were more than five red fruit from each plot, five fruit with the highest a* value and a*/b* were selected. The a* indicates the degree of redness and a*/b* is the ratio of redness to yellowness and is a good indicator of maturity stage (Batu, 2004; Brajovic et al., 2012). The fruit used for carotenoid analysis had an average a* value of 25.4±1.7 and an average a*/b* of 0.95 ± 0.08.

Carotenoid analysis

Sample preparation. On the day of harvest, the tomatoes from each replication were cut from stem to blossom end, and tomato halves were frozen at -20 °C until further analysis. All additional sample preparation and extraction were done under dim light to avoid carotenoid degradation. Prior to carotenoid extraction, the tomatoes were freeze-dried (Harvest Right, Salt Lake City, Utah, USA). The lyophilized tissue was finely ground in liquid nitrogen using a mortar and pestle and frozen at -20 °C until analysis.

Chemicals. All of the chemicals used for carotenoid extraction and determination were high-performance liquid chromatography-grade and purchased from Thermo Fisher Scientific (Waltham, MA, USA). The lycopene and β-carotene standard reference material were purchased from Millipore Sigma (Brulington, MA, USA). The phytofluene standard reference material was purchased from CaroteNature (Münsingen, Switzerland).

Extraction and detection. The extraction and ultra-performance liquid chromatography (UPLC/UV) detection of carotenoids was based on the method by Maurer et al. (2014). A 0.05 g aliquot of the ground lyophilized tissue was added to 6 mL of acetone/methanol (2:1, v/v; 0.5% butylated hydroxytoluene (BHT)) and 3 mL of hexane with 0.5% BHT. The solution was vortexed and then sonicated in an ice bath for 20 minutes. To promote the phase separation, 5 mL of 1 M sodium chloride solution was added. The sample extract was centrifuged at 1800 rpm for 10 minutes at 4 °C (JA-17, Beckman Coulter, Palo Alto, CA, USA). An aliquot from the hexane layer was collected and filtered with 0.22 um, 13-mm PTFE syringe filters (VWR, Wayne, PA, USA) into 2 mL amber sample vials for analysis. The standard reference materials were dissolved and diluted with hexane (0.5% BHT). Stock solutions of the β-carotene and phytofluene were prepared at a concentration of 50 µg/mL, and the lycopene stock solution was prepared at 60 µg/mL. The stock solutions were stored at -80 °C in 2 mL aliquots in amber glass.

Six-point standard curves ranging from 1.875-60 ug/mL for lycopene and 1.5-50 ug/mL for β-carotene and phytofluene were prepared from the stock solutions. The standard curves were filtered into 2 mL amber sample vials for analysis with the samples. New standard curves were prepared for each UPLC analysis day.

A Waters Aquity UPLC equipped with a photodiode array detector (PDA) and a reverse phased HSS C_{18} 2.1 x 100mm, 1.8 µm column were used for sample analysis. The mobile phase A consisted of 75:23:2 acetonitrile/water/hexane (v/v/v, 0.1% acetic acid v/v). Mobile phase B was 90:80:2 acetonitrile/butanol/hexane (v/v/v, 0.1% acetic acid v/v). At a flow rate of 0.5 mL/min, the solvent gradient consisted of a 0.5 min hold on 100% A, a 1 min shift to 0% A, followed by 100% A until 13.5 min. Each sample and standard vial was read in duplicate. Absorbance was recorded at 450, 280, and 350 nm. Lycopene, β-carotene, and phytofluene were identified from their retention time and absorption spectra and confirmed and quantified with the standard reference curves. The carotenoids are expressed on a dry weight basis ($\mu g \cdot g^{-1}$).

Statistical analysis

Statistical analysis was performed using Statistical Analysis Software (SAS version 9.4; Cary, NC, USA). The three compound concentrations—lycopene, β-carotene and phytofluene—were natural log-transformed prior to analysis because the distributions were skewed and non-normal. Each compound was analyzed using a linear mixed model with the PROC GLIMMIX procedure that included DDFM-KR in the model statement. The fixed effects were treatment, year, and treatment x year. The blocking factor and blocking x treatment effect were including the random statement, but only included in the final model if the REML variance estimation was above zero. There was no significant treatment x harvest interaction in 2020, so the two harvests were combined for the final model. The treatment LSmean separation was carried out with a

Bonferonni *P*-value adjustment. LSmeans of the transformed data were back-transformed for presentation.

Compositional data analysis of the carotenoids was performed using the statistical language R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) with the "EasyCODA" package (Greenacre, 2018). The data were transformed and expressed by two logratios: 1.) natural log(lycopene/phytofluene) and 2.) natural log(β-carotene/lycopene+phytofluene). The two logratios will be referred to as LR1 and LR2. The logratios of carotenoid compounds were customized in this study so they could be intuitively interpreted and provide more meaningful results in the compositional data analysis of tomato carotenoids (Wood & Greenacre, 2021). LR1 and LR2 were customized based on the carotenoid biosynthesis pathway—where phytofluene is a precursor to lycopene, and both are precursors to β-carotene. The two logratios were then used as the response variables in mixed linear regression models using the PROC GLIMMIX procedure with DDFM=KR in the model statement. The fixed effects were rootstock treatment and year and the treatment LSmean separation was carried out with a Bonferroni p-value adjustment.

Results

Individual carotenoid concentrations

We found no significant year x rootstock treatment interaction effects on the three carotenoids concentrations, so only the main effects of rootstock treatment are assessed (Table 5.1). The rootstock treatments did not significantly influence the individual concentrations of lycopene, β-carotene, or phytofluene, but there was significant year-to year variation in the compound concentrations ($P<0.0001$) (Table 4.1). Lycopene was the most abundant carotenoid found in the samples and comprised 73%-80% of the total carotenoids quantified (Table 5.2). β-Carotene was the second most abundant carotenoid (12-18%), and phytofluene comprised the remaining 7-9% of the total carotenoid content in the fruit (Table 5.2).

Composition of carotenoids

The effect of production year was also significant for the results of the logratios (LR1 and LR2) between carotenoids (Table 5.1). The LR1 values were not significantly impacted by the rootstock treatments, but there were significant rootstocks effects on the LR2 values (Table 5.1). Regarding the LR2, the rootstock 'RST-04-106-T' was significantly lower than the value for the nongrafted tomatoes and those grafted to 'Fortamino' ($P<.05$). This indicates lower relative β-carotene content in relation to the two carotenoid precursors—lycopene and phytofluene. The other four rootstock treatments had similar LR2 values to the nongrafted controls (Table 5.2). Figure 5.1 shows the relationship between the two logratios, which shows a strong negative linear association between LR1 and LR2. The figure also demonstrations the variation in the logratios attributed to year. The tomatoes harvested in 2018 had the lowest LR1 values and highest LR2. The 2019 harvested tomatoes had intermediate logratios, and those harvested in 2020 had the highest LR1 values and lowest LR2. The scatterplot also shows that the variability

between rootstock treatment LSmeans was primarily along the LR2 axis or related to the relative β-carotene in the composition (Fig 5.1).

Table 5.1 Probability values of individual carotenoids and carotenoid logratios from overall ANOVA F-Test of fixed effects: rootstock treatments, production year, and treatment x year interaction.

	Treatment[z]	Year	Treatment x Year
Lycopene	ns	<.0001	ns
β-Carotene	ns	0.0018	ns
Phytofluene	ns	<.0001	ns
LR1[y]	ns	<.0001	ns
LR2[x]	0.0152	0.0075	ns

[z]Nongrafted treatment was 'Tasti-Lee' and the other treatments are the given rootstock grafted with 'Tasti-Lee' as the scion
y Year is the effect of growing season: 2018, 2019, and 2020
xLR1=ln(lycopene/phytofluene)
wLR2= ln(β-Carotene/lycopene+phytofluene)

Table 5.2 The main effects of rootstock treatment and year on the average content and composition of carotenoids identified in 'Tasti-Lee' tomatoes

	µg·g–1 DW[z]			Proportion of total carotenoids			Logratios[y]	
Treatment[x]	Lyco-pene	β-Caroten e	Phyto-fluene	Lycop-ene	β-Carotene	Phytofl-uene	LR1	LR2
			<u>Rootstock main effects</u>					
Nongrafted	493.09	112.61	46.25	0.75	0.18	0.08	2.48	-1.58 a
Maxifort	579.52	106.14	52.80	0.77	0.15	0.08	2.45	-1.84 ab
DRO141TX	524.53	113.28	51.11	0.78	0.15	0.07	2.57	-1.84 ab
Fortamino	472.29	103.08	48.39	0.73	0.18	0.09	2.27	-1.62 a
Estamino	543.92	107.53	53.74	0.78	0.15	0.08	2.48	-1.83 ab
RST04106T	655.83	101.11	54.49	0.80	0.12	0.08	2.59	-2.09 b
P-value[w]	ns	ns	ns	n/a	n/a	n/a	ns	0.015
			<u>Year main effect</u>					
2018	328.65 a	109.82 a	57.03 a	0.66	0.22	.12	2.99 a	-1.39 a
2019	531.39 b	89.93 b	37.43 b	0.81	.14	0.06	2.60 b	-1.97 b
2020	863.15 c	124.75 a	62.30 a	0.82	0.12	0.06	2.99 b	-2.01 b
P-value	<.0001	0.0018	<.0001	n/a	n/a	n/a	<.000	0.0075

zValues are back-transformed log LSmeans

[y]LR1= ln(lycopene/phytofluene), LR2= ln(β-Carotene/lycopene+phytofluene)
[x]Nongrafted treatment was 'Tasti-Lee' and the other treatments are the given rootstock grafted with 'Tasti-Lee' as the scion.
[w]Probability value of overall ANOVA F-test
LSmeans followed by different letters are significantly different according to pairwise comparisons with a Bonferonni *P*-value adjustment

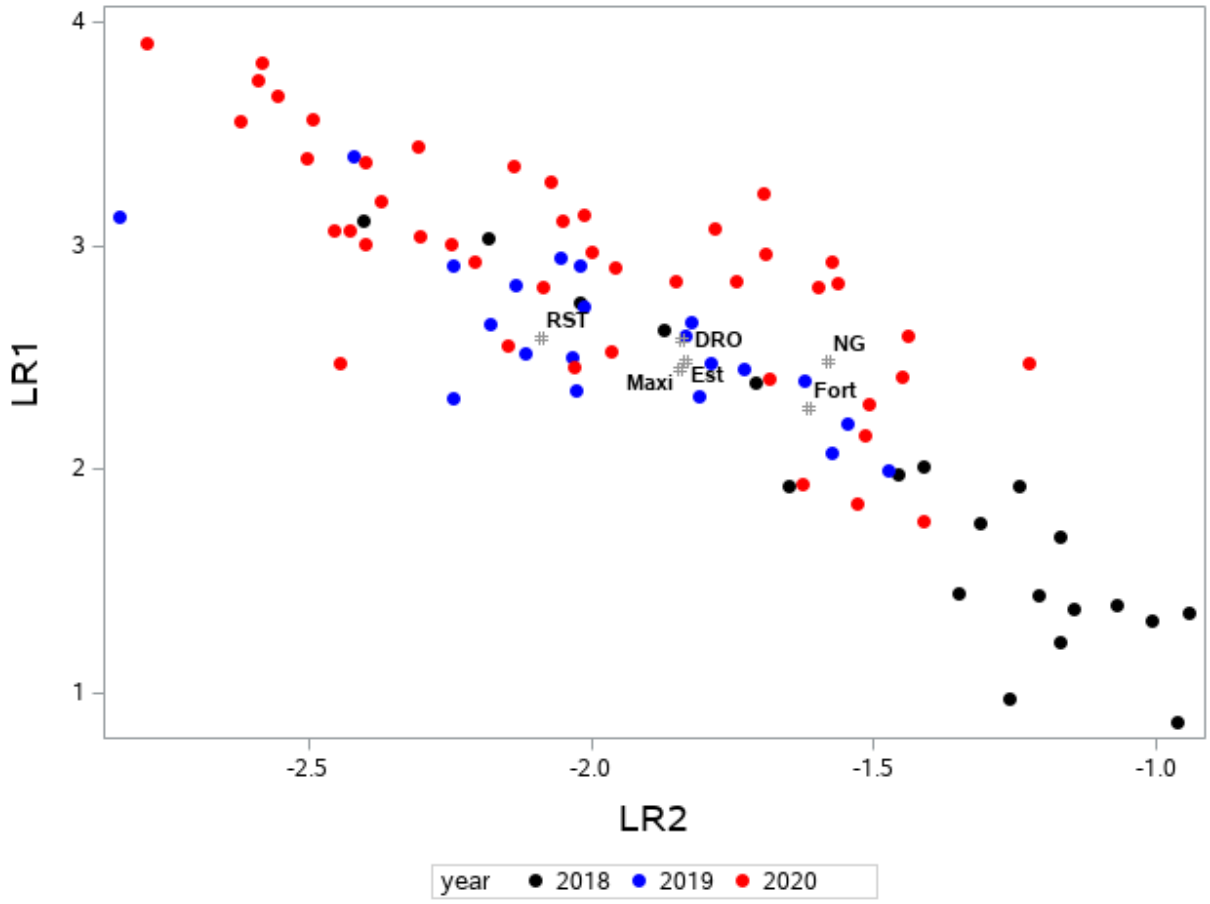

Figure 5.1 The relationship between LR1 (ln(lycopene/phytoflouene)) and LR2 (ln(β-Carotene/lycopene+phytofluene)) of all grafted and nongrafted 'Tasti-Lee' tomato samples harvested in 2018, 2019, and 2020. The LSmean (#) of each rootstock treatment group is identified: NG=nongrafted, Fort='Fortamino', Est='Eastamino', Maxi='Maxifort', DRO='DRO-141-TX', RST='RST-04-106-T'.

Discussion

Consistent with the typical distribution of carotenoids in tomatoes, lycopene was the most abundant carotenoid identified, followed by β-carotene and then phytofluene for all of the grafted and nongrafted plants (Distefano et al., 2020). Grafting did not significantly alter any of the individual concentrations of the identified carotenoids. The plants grafted to 'RST-04-106-T' produced fruit with 33% higher lycopene content than fruit from nongrafted plants, but this effect was not significant.

Other reports on the lycopene concentrations for nongrafted 'Tasti-Lee' are reported on a fresh weight basis (Cantliffe et al., 2009; Ingram, 2020; Scott et al., 2008), so it is difficult to make direct comparisons to the concentrations found in this experiment, which ranged from 472.29-655.83 µg·g–1 DW. Other cultivars containing the og^c gene, have lycopene concentrations that range from 227.11 ± 18.31 µg·g–1 DW (Li et al., 2012) to 4886.6 ± 383 µg·g–1 DW (Vogel et al., 2010). We found that the proportion of β-carotene (12-18%) was lower than that reported for the fresh-eating round tomatoes 'Amati' and 'Gardel', which ranged from 34% to 40% of the total carotenoids (lycopene, β-carotene, lutein) (Brajovic et al., 2012). Lower amounts of β-carotene in relation to lycopene is also consistent for cultivars with the og^c gene because of the defect in β-cyclase, which causes less lycopene to be cyclized to form β-carotene. Interestingly, phytoene—a colorless precursor to phytofluene—was not identified in the chromatographs or was below our detection limit. Other reports have found phytoene in similar or higher concentrations than phytofluene in fresh-eating tomatoes (Distefano et al., 2020; Raffo et al., 2002; Vogel et al., 2010b). These colorless carotenoids are not as often investigated, but there is evidence that phytofluene can contribute significantly to the antioxidant

capacity of fresh-eating tomatoes and processed tomato products (Stinco et al., 2016). Also, phytofluene is readily absorbed by humans (Meléndez-Martínez et al., 2018).

Compositional data analysis and the use of logratios have rarely been used to analyze fruit phytochemical composition. Typically, concentrations of phytonutrients are assessed individually or as non-transformed ratios. The results found in this study show that this kind of analysis can reveal compositional differences between samples subjected to different rootstock treatments, which were not identified in the models of individual compounds This could be attributed to the fact that individual carotenoids do not vary independently. For instance, we found that a higher relative accumulation of lycopene in the samples was associated with a lower relative accumulation of β-carotene. During tomato ripening, lycopene is rapidly accumulated, and the cyclization of lycopene to form β-carotene is reduced (Bramley, 2002).

We found significant year-to-year variation in the carotenoid concentrations and compositions. Different environmental conditions, such as ultra-violet radiation and temperature and truss location and harvest period, can influence the development of tomato carotenoids (Brandt et al., 2006, L. Liu et al., 2015). The sampling dates across the three years were dependent on when the plants produced ample red fruit, which was in October for 2018 and 2019, but was earlier (August, September) in 2020. It would be beneficial for future studies to assess tomato carotenoids in relation to rootstocks across more sampling periods in each year.

All of the rootstocks and nongrafted plants produced fruit with similar LR1 values, indicating that the lycopene to phytofluene ratio was not significantly influenced by the rootstock genotype. The rootstock 'RST-04-106-T' had the lowest β-carotene accumulation relative to the amounts of lycopene and phytofluene in the fruit (LR2). This rootstock treatment was the only one to significantly differ in this parameter compared to the nongrafted plants ($P \leq 0.05$). It is

possible that the rootstock is altering key transcriptional processes related to carotenoid biosynthesis or fruit ripening. Research has shown that watermelons grafted to bottle gourd rootstocks had significantly reduced lycopene content at full maturity compared to nongrafted plant (G. Liu et al., 2016). The researchers identified that nine genes related to lycopene biosynthesis were differently expressed in the grafted plants. (G. Liu et al., 2016).

In this trial we found that the grafting of "Tasti Lee" to the 'RST-04-106-T' produced tomatoes with lower relative β-carotene (12% of total) and higher lycopene (80% of total). Brajovic et al. (2012), used similar methods of compositional data transformations to assess the relationships between lycopene, β-carotene, and lutein of grafted and nongrafted tomatoes through three different ripeness stages. One graft combination ('Amati F1'/ 'Robusta F1') had significantly different logratios of lycopene/β-carotene compared to the nongrafted plants at all three maturity stages. The difference was a result of the grafted treatment having higher β-carotene and lower lycopene which is the inverse of our findings. The rootstock 'Armstrong' also acted to increase the relative amount of β-carotene in 'Sir Elyn' tomatoes passing from the 'breaker' to the 'turning' stage (Mauro et al., 2020). This current study only investigated carotenoid concentrations at the red maturity stage. Still, the rootstock effects found in this study and others warrant further investigation into possible mechanisms of rootstock influence on carotenogeneis during tomato fruit ripening

The other four rootstocks assessed in this study did not differ in the carotenoid composition of red-harvested 'Tasti-Lee' tomatoes. This is consistent with many studies finding no significant grafting effects on carotenoid or lycopene content of tomatoes (Djidonou et al., 2017; Khah et al., 2006; Lang & Nair, 2019; Qaryouti et al., 2007; Turhan et al., 2011). The discrepancy in the grafting effect on tomato carotenoids indicates that this trait is highly

dependent on the rootstock/scion combination. Therefore, it is important to conduct rootstock trials on scions that will be marketed for fruit quality characteristics, such as 'Tasti-Lee'. Here, the only rootstock that impacted fruit carotenoid composition was 'RST-04-106-T', which would not be selected for this production system because it did not improve marketable yields of 'Tasti-Lee' tomatoes (Jenkins, 2021, Chapter 4). While, 'Estamino,' 'Fortamino,' 'DRO-141-TX', and 'Maxifort' all provide marketable yield increases without altering the carotenoid concentrations of this high-lycopene cultivar (Jenkins, 2021, Chapter 4).

Conclusion

The present study aimed to identify the rootstock influence of carotenoid accumulation and composition in 'Tasti-Lee' tomatoes harvested at the red maturity stage. We demonstrated that the concentrations of lycopene, β-carotene, and the colorless carotenoid, phytofluene, were not significantly altered by the five rootstocks that were evaluated. However, we found that one rootstock—'RST-04-106-T'—significantly altered the composition of carotenoids as compared to the nongrafted plants. This alteration was a result of reduced β-carotene accumulation compared to the relatively high lycopene and phytofluene content in the tomatoes. This study is the first to identify and assess the rootstock effects of the minor carotenoid phytofluene in grafted tomatoes. The results reported here suggest that it may be important to determine fruit composition effects in other scions and production systems that use the rootstock 'RST-04-106-T'. Finally, the results indicate that grafting 'Tasti-Lee' to select vigorous rootstock does not compromise carotenoid content or composition.

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Chapter 6-Conclusions and Future Work

This dissertation reported on the results of three independent experiments that aimed to investigate (i) the quality and performance of grafted tomato transplants and (ii) the production performance and fruit quality of grafted tomatoes. The first chapter reviewed the recent literature regarding the rootstock effects on tomato fruit quality. The second and third chapters reported on the transplant quality impacts from two environmental sources of plant quality deterioration (ethylene and heat) during the production and transportation of these plants. The fourth chapter investigated the production performance and fruit quality of 'Tasti-Lee' tomatoes grafted to five different commercially available rootstocks and grown in a high tunnel. Lastly, chapter five assessed the rootstock effects on major and minor tomato carotenoid concentrations and relative carotenoid compositions using compositional analysis techniques.

The literature review found that tomato rootstocks can influence tomato fruit size, texture, color, flavor, and nutrition in both normal and stressed production settings. However, effects on these fruit quality traits are highly subject to specific rootstock-scion combinations as well as interactions with the environment. Often, vigorous rootstocks increase the overall fruit weight or size, decrease fruit SSC, and increase the fruit TA. The alternation of SSC and TA could influence the taste of tomatoes from grafted plants. However, there are no clear trends regarding changes in flavor according to the few quantitative sensory analysis studies that have been conducted on fruit from grafted tomato plants. In regards to changes in fruit nutrients and phytochemicals, vigorous rootstocks have often been associated with lower AsA content in tomato fruit. While, changes in carotenoid concentrations, phenolic compound concentrations, and fruit antioxidant capacity are more wide-ranging and subject to rootstock-scion combination.

The results from the second chapter indicate that exogenous ethylene exposure at 1 $\mu L \cdot L^{-1}$ or above can be a significant source of grafted tomato transplant quality deterioration. Four days of high ethylene concentrations resulted in physiological stress (F_v/F_m) in both grafted and nongrafted plants. However, the grafted plants better maintained the F_v/F_m, indicating and improved tolerance to prolonged ethylene exposure. Ethylene damage also resulted in lower leaf necrosis, abscission of seedling cotyledons, and reduced plant compactness in grafted and nongrafted plants. The shoot biomass was significantly lower during the first two weeks of growth of ethylene-damaged plants compared to nongrafted plants, but there were no significant plant growth reductions by the third week of growth. These results suggest that propagators of grafted tomato transplants should take persuasions to avoid accumulation of ethylene above 1 $\mu L \cdot L^{-1}$ in the greenhouse, during packaging, and during transportation to ensure that the plants remain salable and that their customers are satisfied.

Our results from the third chapter indicate that short-term exposure to moderate heat stress (32.8 ± 0.17 °C) temperatures during transportation of grafted tomato seedlings is also a source of plant quality declines. The grafted and nongrafted plants experienced dose-specific reductions in F_v/F_m from the high-temperature exposure. The non-ideal transportation temperatures also limited transplant quality of grafted and nongrafted plants by reducing shoot biomass and plant compactness. However, early growth was not significantly impacted. These results suggest that shipping grafted seedlings for up to three days without temperature control is feasible if outdoor temperatures are not excessively warm (above 35 °C). Considering the results from chapter 1 and chapter 2, future work on the quality of grafted seedlings would benefit from a better understanding of acceptable and non-acceptable plant symptoms from growers

purchasing these higher-priced plants. A visual quality scale could be developed that reflects the point of salability of the seedlings.

Our results from the forth chapter indicates that rootstocks ‘Maxifort’, ‘DRO-141-TX’, ‘Estamino’, and ‘Fortamino’ increased the marketable kgs harvested per plant by 31.5%-47%, the average fruit weight by 11%-15% and the number of extra-large fruit harvested per plant compared to nongrafted ‘Tasti-Lee’. The rootstock ‘RST-04-106-T did not improve the yield of ‘Tasti-Lee’ tomatoes, but plants grafted to this rootstock did produce fruit with significantly higher SSC compared to fruit from plants grafted to the rootstock ‘Maxifort’. No other fruit quality traits (TA, SSC/TA, lycopene content, antioxidant capacity, ascorbic acid content) were influenced by the rootstocks assessed. From this chapter, we can conclude that the premium cultivar ‘Tasti-Lee’ could fit in well to high tunnel production systems when grafted to select vigorous rootstocks without compromising fruit quality.

The fifth chapter found that the concentrations of lycopene, beta-carotene, and phytofluene of mature red ‘Tasti-Lee’ tomatoes were not significantly influenced by five rootstocks. However, one rootstock—‘RST-04-106-T’—did significantly alter the relative composition of tomato carotenoids compared to fruit from nongrafted plants. Fruit from this rootstock treatment had a lower relative composition of beta-carotene in relation the relatively high lycopene and phytofluene content in the tomatoes. These results indicate that rootstock genotype could influence carotenogenesis during tomato fruit development.

Future work should focus on identifying the underlying mechanisms of rootstock effects on fruit traits. There is evidence from the literature review and the final chapter of this dissertation that rootstocks may influence the ripening patterns and/or carotengenesis in tomatoes. Therefore, it needs to be determined if the measured differences in primary and

secondary metabolites is due to actual accumulation differences or due to varied maturity stages between fruit from grafted and nongrafted plants. Alternatively, it is suggested that altered sink/source relationships in grafted plants with high fruit loads and greater root and vegetative biomass could cause the changes in fruit composition.

Changes in fruit composition may also be attributed to changes in vascular transport of hormones, mRNA, and mobile proteins between the rootstock and scion. There is a need for transcriptomic and proteomic methods to elucidate the molecular mechanisms for the vascular transport of macromolecules and signal substances between rootstocks and scions. Identifying relevant DEGs or differently expressed mRNA would help unravel scion-rootstock and scion-rootstock-environment interaction influences on tomato fruit quality traits. Studies utilizing both metabolomics and transcriptomics methods in grafted *Cucurbitaceae* species have identified altered expression of fruit quality-related traits such as primary metabolites, phytohormone signaling and fruit ripening

In tomatoes, comparative transcriptomics has helped identify molecular mechanisms of grafting-induced tolerance to abiotic stress resistance (Ntatsi et al., 2017) and virus infection (Spanò et al., 2020). Future transcriptomics work in grafted tomatoes should identify possible mechanisms for fruit quality impacts, especially related to changes in fruit ripening, hormone signaling, and production of primary and secondary metabolites. Lastly, given that tomato rootstock breeding is generally focused on agronomic traits, disease resistance, and stress tolerance, the use of grafting to improve the organoleptic or nutritive value of tomatoes has not been widely investigated. There is evidence that further use of related wild *Solanum* species in tomato rootstock development could help identify lines that improve fruit quality in normal or stressed conditions.